

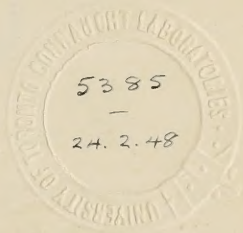
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COLLECTED PAPERS

from

THE RESEARCH LABORATORY PARKE, DAVIS & CO. DETROIT, MICH.

DR. E. M. HOUGHTON, Director



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**Studies from the Research Laboratory.
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**GASTRO-INTESTINAL LAVAGE IN DOGS; ITS VALUE
IN REMOVING WORMS AND IN OTHER
RESPECTS.**

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From evidence obtained in conversation and correspondence, it appears that the use of gastro-intestinal lavage, washing out the digestive tract with a stream of water administered by rectum as an enema and continued until it issues from the mouth by repeated emesis, for the removal of worms from dogs and cats, is rather common practice among veterinarians in this country. In spite of this, there seem to be almost no published references to the practice and it is unknown to many veterinarians of long standing. It has therefore seemed worth while to conduct some investigations in regard to this form of lavage in connection with some anthelmintic investigations, to ascertain the efficacy of this mechanical method of removing worms in comparison with the customary medicinal procedure, and also to note its immediate and remote effect on the host animal and its possibilities as a mode of administering medication.

Sisson (1914) states regarding the digestive tract of the dog: "There is an ileocolic valve. * * The valve does not seem always to be efficient, since experience shows that rectal injections can be made to pass beyond it. This may be partly due also to the fact that the terminal part of the ileum runs horizontally forward, and its orifice faces forward into the beginning of the colon."

It appears that the corresponding valve in the cat is likewise pervious, since fluids may pass from the rectum and colon to the small intestine, and it may be that this is generally true of carnivores. This is practically the limit of applicability of the method of gastro-intestinal lavage, as there are obvious anatomical, physi-

ological, practical or esthetic objections to its use in the case of such animals as horses, cattle, sheep, hogs or man.

Quitman (1917) describes and discusses the method of gastro-intestinal lavage as follows:

"In the dog, I vary my vermifuge treatment. In my own hospital practice, we usually make very quick and thorough work with gastro-intestinal lavage. The method works so well that we are getting a special clientele on that score alone. We first administer a thorough purgative, preferably a saline, to empty the bowels, of course, as much as possible. Then with just ordinary soap solution, using several gallons, we first free the bowels of all material by letting a certain amount run in and letting it be ejected again, and finally after a little while, especially helped by elevation of the hind quarters of the dog, the fluid will come out of his mouth as fast as it runs into the rectum. This is the most efficacious method, as it will wash out every worm in the dog; it tends to wash out the ova and also free the system of the excretions of the worms that so often seem to cause various forms of toxemia. After flushing the dog with the soap solution, we usually flush him freely with a normal salt solution, expecting some of that to remain, which will tend to ward off the slight prostration that sometimes results from the operation. If the dog is very weak, we postpone the gastro-intestinal lavage until we get him braced up."

In response to a personal letter from one of us (Hall), Doctor Quitman very kindly furnished the following additional information in regard to this treatment:

"In regard to the gastro-intestinal lavage treatment for the removal of parasites, I have never seen the method in print. I first saw it used by Dr. Charles A. White of 216 East 26th St., Chicago, professor of canine medicine and surgery at the Chicago Veterinary College, about 15 years ago. It is without a doubt the safest, surest method (when done correctly) of removing all varieties of intestinal parasites. I have used the method ever since I first saw White demonstrate it. I use it in my hospital at least 25 or 30 times monthly, counting individual dogs and not repetitions (I like to give two treatments, three or four days apart).

I prefer purging the animal first, but circumstances do not always permit this preliminary. If not, we gradually wash out the intestines, allowing the animal to force out the water and feces

before filling the bowels and elevating the hind part of the body and preventing back expulsion by pressure on anus. One case in 100 may be found difficult; when so, administer 1/20 to 1/10 gr. apomorphine hyd. and again fill them up, when the fluid will come through. I use sometimes a gallon of warm soap emulsion, always followed up with a gallon of warm normal salt solution, and sometimes only the normal salt solution.

"It also operates well in cats. * * * Dr. White may be able to give you more of the history, as I do not know whether he originated the idea or not."

We are unaware of any European work, or work elsewhere outside of the United States, dealing with this topic, but we have made no effort to ascertain what literature of the sort may exist.

Our own experiments with this method comprise washing out the gastro-intestinal tract in various ways, examining the material washed out, and usually keeping the animals for some days and examining all feces or vomitus for worms that might have been unfavorably affected by the lavage and subsequently passed. The injection of the water was accomplished by gravity—using a funnel and tube—or by a force pump or city water pressure. The dogs were killed at the end of the experiments and examined for worms and for pathological lesions, especially those of the digestive tract. A detailed statement of the experiments is as follows:

Dog No. 114, a mongrel weighing 4.5 kilos, was washed out with an indefinite amount of tap water, no worms being discovered in the water vomited by the dog. On postmortem examination 3 days later, the dog was found to have 3 *Dipylidium* and 1 whipworm. The gastric mucosa was slightly paler than normal. The treatment appeared to be entirely ineffective in this case, and apparently uninjurious to the animal.

Dog No. 124, a small wire-haired terrier, was washed out with an indefinite amount of tap water; no worms were found in this water. The dog was killed immediately after treatment and found to have 1 ascarid and 1 whipworm. The entire digestive tract appeared anemic and edematous as a result of the washing. The treatment was entirely ineffective.

Dog No. 125, a small terrier, was washed out with an indefinite amount of tap water. No worms were removed and none were found on postmortem examination immediately after treat-

ment, so no conclusions could be drawn regarding efficiency. However, the abdominal cavity was found to contain a blood-stained fluid, and the origin of the blood was found in 2 large ruptures of the jejunum and one of the colon, and 3 lesser breaks in the colon. The large breaks were an inch long or longer. All ruptures involved the serosa and the muscular coat, but the mucosa was not apparently broken. The intestine also showed numerous submucous hemorrhages. The damage was obviously due to the pressure of the water, and it is evident that there is a certain danger here. Should the initial washing out of the large intestine fail to remove all hard fecal masses, one of these might easily occlude the ileocolic valve and lead to rupture of the large intestine as a result of the back pressure, and food masses, hair balls, worm knots, etc., in the stomach might occlude the esophageal aperture, leading to rupture of the coats of the small intestine or the stomach. A spastic condition of the ileocolic valve or the pylorus, or a moderate degree of stenosis at some point, might give similar results.

Dog No. 126, a small terrier, was washed out with an indefinite amount of tap water. The washing removed 1 ascarid, and on postmortem immediately afterward the dog was found to have one more. The treatment was therefore 50 per cent effective against ascarids. The entire digestive tract was anemic and edematous as a result of the washing.

Dog No. 119, a mongrel weighing 15.5 kilos, was washed out with 8 liters of soap solution, followed by a half liter of normal salt solution, and this in turn followed by a considerable, but unmeasured, amount of tap water. Six hookworms were washed out in this way. One hookworm was passed in the feces collected on the third day, and on postmortem examination the third day another hookworm was found in the large intestine, dead and being passed out. This total of 8 hookworms was credited to the efficacy of the lavage. However, the dog had 157 hookworms and 54 whipworms in addition. The treatment was therefore 5 per cent effective against hookworms and 0 per cent effective against whipworms. The stomach was normal; the small intestine showed petechiae due to hookworms; the colon showed one large, discrete inflamed area and one or two small hemorrhagic areas; the tip of the cecum, covering the area from which

the whipworms were removed, was moderately inflamed, a lesion which we commonly find associated with, and evidently due to, whipworms. There was evidently a complete recovery from the anemic edematous condition produced by the lavage (it would be expected that this condition would be transitory), and it may be doubted whether any of the lesions noted could be attributed to the treatment.

Dog No. 121, a mongrel weighing 12.75 kilos, was washed out with 8 liters of soap solution followed by a half liter of normal salt solution. No worms were washed out and none passed during the next 8 days. The dog was then treated with oil of chenopodium, but passed no worms during the next four days. Post-mortem examination showed the presence of 28 whipworms. The lavage, as well as the oil of chenopodium, was 0 per cent effective against the whipworms. The digestive tract was normal.

Dog No. 128, a bulldog weighing 10 kilos, was washed out with an indefinite amount of soap solution at the clinic at the joint meeting of the Michigan and Northwestern Ohio Veterinary Medical Association of 1917. No worms were removed by the washing. Postmortem examination immediately after treatment showed the presence of a number of hookworms and *Dipylidium*, the number not being counted owing to the limited time and facilities. The treatment was 0 per cent effective against hookworms and *Dipylidium*. The small intestine showed petechiæ due to hookworm and a slight inflammation of the ileum; aside from this, the only thing noted was the edematous condition of the digestive tract as a result of the washing, a condition which, as already noted, is probably transient.

Dog No. 209, a mongrel weighing 9 kilos, was treated by gastro-intestinal lavage, using 8 liters of water. The lavage washed out 2 entire specimens of *Tania pisiformis*. The dog was killed the next day and found to have 7 specimens of *T. pisiformis*. The lavage was therefore only 22 per cent effective against *Tania*. There were numerous petechiæ in the cardiac and pyloric ends of the stomach, possibly due to the strain of vomiting. The small intestine was mildly inflamed, except where the tapeworms lay in the jejunum, and here the mucosa was highly inflamed and showed some hemorrhage.

The following experiments involve the use of gastro-intestinal lavage, but are complicated by other features.

Dog No. 135, a mongrel weighing 9 kilos, was washed out with 200 mls of a 1 per cent solution of copper sulphate, then with an indefinite amount of tap water, then with another 200 mls of copper sulphate solution, then with an indefinite amount of tap water again. The treatment brought away 1 headless *Dipylidium* stained blue with copper sulphate; no more worms were passed and the dog was found free from worms on postmortem examination the fourth day after treatment. The treatment was therefore 100 per cent effective against *Dipylidium*. However, the addition of the copper sulphate complicates the case, as it is no longer a mechanical removal of the worm which is involved, but a medicinal treatment which is given by rectum instead of by mouth, merely a modification here of the common rectal medication. Copper sulphate is well known as an anthelmintic for use against stomach worm in ruminants; it cannot be given by mouth to dogs for this purpose, owing to its decided emetic action. In this experiment, the copper sulphate also gave rise to serious lesions. The entire digestive tract was inflamed and hemorrhagic, the liver cirrhotic, and the kidneys were inflamed and showed what were apparently streaks of degeneration. The lesions of the digestive tract, at least, must be attributed to this treatment. The hemorrhagic condition of the digestive tract and the resultant blood in the feces, with the dog in a weakened condition, led to the dog developing a case of rectal myiasis from infestation with blowfly larvae. This was successfully treated with a rectal injection of 25 to 30 mls of olive oil containing 30 per cent chloroform, and none of the larvae were found postmortem.

Dog No. 187, a mongrel weighing 10 kilos, was given 6 grains of calomel the day before treatment. The day of treatment the dog was given a rectal injection prepared by dissolving 2 grams of beta-naphthol in 9 mls of 95 per cent alcohol and adding this to 1000 mls of water to give a finely divided watery suspension of the drug. The day of treatment the calomel had failed to purge the dog, so the rectum and colon were thoroughly flushed out with tap water, and then the rectal injection of beta-naphthol was given. Evidently the amount given was too much for the dog's large intestine, and though it was not intended as gastro-

intestinal lavage, the animal vomited a half-hour later, vomiting a headless *Dipylidium*, indicative of the passage of the drug into the small intestine. The dog passed no feces the next 3 days and died on the fourth or fifth day. There were no worms found postmortem, so the treatment was 100 per cent effective against *Dipylidium*. The stomach was inflamed and catarrhal and contained fecal matter; the intestines were inflamed. Like the previous experiment, this is really rectal medication, not gastro-intestinal lavage, and while effective against *Dipylidium* it exerted a bad effect on the digestive tract.

Dog No. 172, a mongrel weighing 10 kilos, was given an enema in order to secure a fecal sample to examine for the presence of parasite eggs. This is a routine procedure with us; a piece of flexible rubber tubing about 3 feet long and approximately a half inch in outside diameter is connected to a tap and the hot and cold water mixed to give a moderate-sized, warm stream. In this case it would appear that the enema had been continued until the water entered the small intestine, for presently the dog passed the fluid and it was found to contain about 20 entire *Dipylidium*. While these worms might have been in the large intestine on their way out, we assume that they were not. The dog was then given a lethal dose of oleoresin of male-fern as a check on some other tests. The next day the dog was very sick. It had occurred to us, and Dr. Horace Hoskins made the same point in conversation with one of us (Hall), that this lavage might be of value in cases of poisoning, so we used the lavage treatment on this dog, though the long time since the male-fern had been given made it unlikely that lavage would be of value. The dog died the evening of the fifth day after treatment with male-fern. Postmortem showed the absence of any parasites, so the unintentional lavage effect—an enema carried to the point where it entered the small intestine and then passed out again by way of the rectum instead of the mouth—was apparently 100 per cent effective against *Dipylidium*. The failure to be of value in poisoning in this case is readily explained by the long interval between the administration of the poison and the lavage.

The value of lavage in cases of poisoning was tested as follows:

Dog No. 204, a terrier weighing 9.5 kilos, was given 20 mils

of oleoresin of male-fern, a lethal dose. The dog had a temperature of 104° at the time and showed indications of distemper. One hour after treatment the dog was given gastro-intestinal lavage, using about 6 liters of water, which washed out quite a little male-fern, and incidentally brought away 3 ascarids. The next day the dog was weak and progressive emaciation was noticeable during the next few days. The dog died the seventh day after treatment. Postmortem examination showed the patchy pneumonia of distemper and a hemorrhagic gastritis and enteritis. No parasites were present, so the combination of male-fern in lethal dose and lavage was 100 per cent effective against ascarids. The lavage failed to save the life of the dog, but the failure was very likely due to the fact that this dog was sick with distemper before treatment, and that the weakened and diseased organism did not have the resistance and vitality to endure the local irritation and toxic effects of the male-fern or the shock of lavage. There was more or less likelihood of the animal dying from distemper, even without the administration of male-fern, and in our opinion the use of lavage within an hour or so after the administration of a lethal dose of male-fern to a healthy dog would usually save the animal. Even in this case, the dog did not show the trembling which we usually find in dogs suffering from the toxic effects of male-fern, and the gastro-intestinal hemorrhage, due in part, at least, to the male-fern, was the principal injurious action of the drug. Another test of lavage in poisoning was as follows:

Dog No. 203, a terrier weighing 10 kilos, was given 1 grain of strychnine sulphate in the form of a powder placed on the back of the tongue and washed down with a little water. Gastro-intestinal lavage was then begun immediately with a rubber tube attached to a tap, and the dog vomited the first of the injected water about 5 minutes after the strychnine was swallowed. About 4 liters of water were vomited by the dog, incidentally bringing away 15 *Dipylidium*. About a half hour later, the dog pitched forward in the characteristic tonic spasm of strychnine. While still rigid, the animal was picked up and filled full of cool water by rectum till it vomited once, on the theory that the cool water filling the digestive tract might exert a depressant effect which would counteract to some extent the stimulant effect of the

strychnine. The rigidity soon passed away and the animal showed no more bad effects from the strychnine. The next day, the dog was running about and eating heartily. The dog was killed on the seventh day after treatment and showed a normal digestive tract and no lesions attributable to the strychnine or the lavage. There were, however, 95 *Dipylidium*, many of them being large, complete strobilæ. The lavage was therefore only 14 per cent effective against *Dipylidium*. The dose of strychnine given was three to ten times that regarded as a lethal dose, and there is no question but what the use of lavage saved the life of the dog in this instance. Of course, in practice a veterinarian could not get to a dog given a grain of strychnine in time to save it by lavage or any other method, but where a poison is still present in the stomach, the use of lavage appears to be one of the first things indicated. It is likely that the simultaneous use of apomorphine hydrochloride, $1/5$ to $1/10$ of a grain, subcutaneously, would be of value in this connection, but the apomorphine alone does not seem to us as useful as the combination, because it is possible for drugs of varying physical composition and consistency to adhere to the stomach walls or lie along the greater curvature in such a way that mere vomiting will not remove them, and actual washing with large amounts of water, thereby dissolving the drugs or forcibly washing them out, is called for. It is also known that apomorphine may fail to produce emesis in narcotic poisoning.

In passing, it might be noted that dogs usually offer but little resistance to gastro-intestinal lavage, and that those which are at first inclined to resist become very subdued during the course of the operation.

An examination of the results in the tests of lavage alone in the foregoing shows: Five dogs were washed out with a plentiful, but unmeasured, amount of tap water, and killed immediately after treatment; the treatment was 0 per cent effective against ascarids once, against hookworms once, against whipworms twice, against *Dipylidium* twice, was 50 per cent effective against ascarids once, and in one case the absence of worms prevented any conclusions. Two dogs were washed out with 8 liters of soap solution, followed by a half liter of normal saline, and in one of these cases this in turn was followed by an indefinite amount of tap water; one of these dogs was killed on the third day and the

other on the twelfth day after treatment; the treatment was 0 per cent effective against whipworms in both cases and 5 per cent effective against hookworms in one case. Another dog was washed out with 8 liters of tap water; the dog was killed the next day and the treatment found to be 22 per cent effective against *Tenia*. One dog was given an enema with an indefinite amount of tap water; the dog died the fifth day after and the treatment found to be apparently 100 per cent effective against *Dipylidium*. Another dog was washed out with 4 liters of tap water; the dog was killed 7 days later and the treatment found to be 14 per cent effective against *Dipylidium*. These tests on 9 dogs show a percentage efficacy against various species of worms of zero in 8 instances, of 5 in 1 instance, of 14 in 1 instance, of 22 in 1 instance, of 50 in 1 instance, and of 100 in 1 instance. The fact that the efficacy is zero in 8 out of 13 instances, and below 50 per cent in 11 out of 13 instances, indicates rather definitely that this treatment has limited anthelmintic value.

The 2 cases where drugs were added to the lavage must be considered rectal medication and disregarded, so far as the treatment called gastro-intestinal lavage is concerned, and the treatment combining lavage and male-fern must also be disregarded in the case of dog No. 201, so far as the efficacy of simple gastro-intestinal lavage is concerned.

It will be noted that all our tests deal only with a single lavage treatment, which we regard as a fair test, although Doctor Quitman states that he likes to give repeated treatments. In this connection we also note the following statement by Quitman (1917):

"I have maintained for many years that this thing of giving one or two doses of a vermifuge or vermicide and then considering the patient free from worms, is an error. We should do it, so as to relieve the animal as quickly as possible of the multitude of parasites that he perhaps has in him, but there is no vermicide that destroys the ova. What are we going to do as these ova hatch out? Oddly enough, according to my observations, it seems that adult worms succumb more readily to vermicides than do young worms."

We are in entire agreement with Doctor Quitman regarding the inadequacy of considering a patient free from worms because anthelmintic treatment has been given, or even, we might add,

because worms have been passed after treatment. We are likewise of the opinion that young worms are more resistant to anthelmintic treatment than are mature worms, and might add further than we agree with Railliet (1915) that large worms are apparently more vulnerable than small ones. But we do not regard the fact that anthelmintics are innocuous to worm eggs as an argument in favor of gastro-intestinal lavage or against anthelmintics in single or repeated doses. Peristalsis alone will remove these eggs, which will require a certain length of time for development before they are capable of infecting another dog, and the disposal of this infective material is a problem in sanitation, not a matter of anthelmintics or lavage.

The use of gastro-intestinal lavage has raised in our mind some questions as to the gastric capacity of the average dog, the gastric capacity per kilo of weight of dog, and, incidentally, the weight of the average dog. These are matters of interest in comparing anthelmintic treatments of the dog and of man.

In the work on anthelmintics by Hall and Foster in the U. S. Bureau of Animal Industry and by Hall at Detroit, the weight of the average dog has been arbitrarily regarded as 10 kilos (22 pounds), with the idea that this was very close to the correct weight and was a convenient figure on which to compute doses. There are now available to us here the weights of 200 dogs on which to base an estimate of the average dog's weight. These dogs were from the Detroit city pound and may be regarded as representative. They do not include the toy varieties on the one hand nor the largest breeds such as the Great Dane, St. Bernard, or Newfoundland on the other, two groups which may be roughly regarded as offsetting one another. The smallest dog in the series weighs 3 kilos and the largest 25 kilos. The average weight is 10.57 kilos. The figures warrant the designation of 10 kilos as the weight of the average dog. A graph indicates that the peak for number of dogs of given weights, also, is reached around 10 kilos.

Sisson (1914) says of the dog:

"The stomach is relatively large. Its capacity in a dog weighing about 40 pounds is about 6 to 7 pints. Colin estimates the average capacity at about 3 liters (ca. $6\frac{1}{2}$ pints), with a range between 0.6 and 8 liters (ca. $1\frac{1}{3}$ to $17\frac{1}{2}$ pints). Neumayer gives the capacity as 100 to 250 c.c. per kilogram of body-weight

(ca. 2.7 ounces per pound). The average capacity of the human stomach is estimated at 1.2 liters ($2\frac{1}{2}$ pints)—not much more than a third of that of a dog of medium size.”

Our own figures are based on the measurement of the stomachs of 25 dogs. In one or two cases, these stomachs were from dogs which had been dead a matter of hours, the stomachs being cold, but elastic and substantially as easily measured for capacity as the stomachs of freshly killed dogs. The other stomachs were from freshly killed dogs. The stomachs were detached at the entrance of the esophagus and at the pyloric aperture and were filled with water until the stomach was full and distended with the weight. This water was then poured into a large graduate and measured. The table of weights and measurements is as follows:

Dog No.	Capacity in mls.	Weight in kilos.	Capacity per kilo.	Dog No.	Capacity in mls.	Weight in kilos.	Capacity per kilo.
175	560	19	29.5	196	1085	9.5	114.2
177	1140	15	76.0	197	3100	17	182.4
183	1190	11.5	103.5	198	475	10	47.5
184	160	5.5	29.1	200	1300	18.5	70.3
185	260	8.5	30.6	201	1200	11.5	104.3
187	460	10	46.0	209	500	9	55.6
188	470	9	52.2	208	475	9.5	50.0
190	1160	11	105.5	207	370	10.5	35.2
191	810	8.5	95.3	205	880	7.75	113.5
192	840	10.5	80.0	206	780	9	86.6
193	850	9	94.4	203	1250	10	125.0
194	1860	13	143.1	204	460	9.5	48.4
195	1210	10	121.0				

The average stomach capacity for these 25 dogs is 913.8 mls, and the average capacity per kilo of weight of dog is 81.6 mls. The smallest stomach capacity, 160 mls, and the smallest capacity per kilo, 29.1 mls, are those of the smallest dog, No. 184, weighing 5.5 kilos, the stomach of which was cold when measured, the dog having been found dead instead of being freshly killed as in the case of nearly all the other dogs. The largest stomach capacity, 3100 mls, and the largest capacity per kilo, 182.4 mls, are those of dog No. 197, weighing 17 kilos. It will be noticed that this is not the largest dog: Dog No. 200, weighing 18.5 kilos, had a stomach capacity of 1300 mls and a capacity per kilo of 70.3 mls, while the largest dog, No. 175, had the surprisingly small stomach capacity of 560 mls and a capacity per kilo of only 29.5 mls.

Taking the average weight of a dog as 10 kilos, an assumption warranted by our weights of 200 dogs, and the average capacity

per kilo as 81.6 mils, as determined above, the average capacity of a dog's stomach would be 816 mils. Taking this in connection with our findings of 913.8 mils as the average capacity for 25 dogs, we are of the opinion that for working purposes one may assume that the average dog weighs 10 kilos and has a stomach capacity of 1 liter. Our figures indicate that it would be actually smaller than 1 liter. In the quotation from Sisson (1914) it will be noted that the average capacity of the human stomach is estimated at 1.2 liters. We find that some texts on human anatomy state the capacity of the stomach of the human male as 2.5 to 4 liters. It would appear, therefore, that the stomach of the average dog is smaller, not larger, than that of the average person.

For the convenience of those unaccustomed to the metric system, it may be stated that a kilo, or kilogram, is equivalent to 2.2 pounds, and that a liter is equivalent to 2.1 pints.

Summary. Experimental investigations of various procedures which may be regarded as gastro-intestinal lavage in the sense in which the term is now used among American veterinarians, or some modification of that procedure, have been made by us in 12 cases. We conclude from these tests:

1. That gastro-intestinal lavage, like most of the medicinal anthelmintics, has not the entirely dependable efficacy which is usually credited to it by those who use it.

2. That it has a certain limited efficacy in removing worms and that it might be of value in anthelmintic treatment in one of several ways; by mechanically removing part of the worms present at times, or all of them less frequently, by removing material that interfered with the action of medicinal anthelmintics, or by employment as a means of administering medicinal anthelmintics in properly selected cases. Its anthelmintic value is less than that of properly selected medicinal anthelmintics in suitable doses.

3. That it probably has greater value in cases of poisoning in dogs or cats, animals which are often poisoned, where the lavage can be given in time to wash out some or all of the poison from the digestive tract before the absorption of a lethal dose. Where it could be applied promptly we would regard it as a procedure of great value.

4. That it is temporarily very depressing and may at times

cause rupture of the intestinal walls from the water pressure, or hemorrhage of the stomach from protracted forcible emesis.

5. That the treatment is easy and rapid with some dogs, but slow and tedious with others.

Investigations based on the weights of 200 dogs indicate that for practical purposes the weight of the average dog is about 10 kilos; investigations based on the above and on the measurement of 25 dog stomachs indicate that the gastric capacity of the average dog is about 1 liter and that the gastric capacity of the average dog per kilo of body weight is about 100 mls. The exact computed weight is a little over 10 kilos and the exact computed capacity and capacity per kilo are a little less than these figures.

REFERENCES.

- QUITMAN, E. L. 1917. A course of treatment necessary for parasitisms. *Am. Jour. Vet. Med.*, v. 12 (5), May, pp. 299-300.
 RAILLIET, A. 1915. L'Emploi des médicaments dans le traitement des maladies causées par des Nématodes. *Rec. d. Méd. Vet.*, Paris, v. 91 (15), 15 Août, pp. 490-513.
 Sisson, SEPTIMUS. 1914. The anatomy of the domestic animals. Philadelphia and London. 930 pp., 725 figs.

**Studies from the Research Laboratory.
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THE TREATMENT OF BURNS BY THE APPLICATION OF PARAFFIN.

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Protective substances in the treatment of burned or severely irritated tissue areas have been recognized as an important therapeutic means of handling a most discouraging surgical condition for many years, and the wide diversity of end results has tended to keep the subject before surgical research bodies in some degree much of the time.

Oils, chemicals and emollients, plastic applications, thermic air baths, under-water treatments, and wax, have all been experimentally tried, adopted for a moment, so to speak, and as quickly laid aside because of the unsatisfactory and inconsistent results obtained.

Recent literature is replete with information concerning the treatment of burns with paraffin preparations. While many good results are to be found among cases which have been treated by almost any of the above mentioned methods, none have shown the consistently satisfactory results which paraffin application has produced, though even in this treatment the final effect varies with certain factors, which it is the intent of this paper to show.

The action of most of these substances is based upon the purely mechanical properties of the paraffin base (Hull). It would seem, therefore, that if such is the case and the results heretofore obtained are really as good as reports credit them with being, a substance having certain active therapeutic properties would be even more brilliantly successful. Experiments hereinafter quoted show this to be true. Of the work already accomplished by paraffin substances little need here be said, since it is the subject of innumerable reports in other periodicals. The conclusions already drawn from these experiments show that the action of the better known paraffin preparations is as follows:

1. Mechanical, consisting of (a) splint-like control of local nervous reactions; (b) protection of wound from air and external influences.

2. Apparently therapeutic: (a) Encouragement of new granulations; (b) analgesic; (c) cicatrization greatly eliminated; (d) grafting of skin often unnecessary.

Beiter attempts to prove by certain experiments with paraffin immersed in water that the ingredients held in the paraffin base are released only in infinitesimal amount, and after two and a half hours fail to show any accumulation in the liquid. However, specimens immersed in serums and saline solutions tend to show that while the amount released from the paraffin is comparatively low, there is sufficient to promote therapeutic reactions which are very desirable and a distinct advantage in the product.

These experiments follow:

Experiment No. 1.

A paraffin mixture "G" *infra*, containing salicylic acid, was immersed in normal horse serum and let stand at room temperature for two hours. At the end of fifteen minutes a strong ferric chloride test reaction was obtained which steadily increased during the two hours, although the container was undisturbed and no effort at shaking down the substance was made. It was then incubated at 37° C. for twenty-four hours, and occasional observations proved that considerable salicylic acid was released at each reading. The paraffin when removed to another vessel at the end of twenty-four hours still released some acid, but the action of the fluids upon the paraffin was not thoroughly tested. The external surface of the paraffin appeared somewhat granular.

Experiment No. 2.

Paraffin mixture "E," to which methylene blue was added, was immersed in serum as before, and placed at room temperature for two hours. At the end of one hour the serum was a distinct blue, which increased proportionately during the succeeding hour, when the container was placed in the incubator at 37° C. This experiment showed conclusively that the paraffin continued to release the blue for several hours, and that the

material released was sufficient to have some medicinal effect was illustrated by the following experiment.

Experiment No. 3.

Saline solution to which methylene blue had been added by immersion of preparation used in Experiment 2 was added, and was injected subcutaneously in a dog weighing 8 kilogrammes. At the end of three hours a distinct blue was noted in the urine. The experiment was also tried by laying a piece of paraffin in the subcutaneous tissue and closing the wound with sutures. Staining of the adjacent tissues was also sufficient to demonstrate histological structure. The appearance of the paraffin showed whitened granular surfaces and a somewhat deeper exudative process than might be expected. It is evident that the area of the exposed surface is, however, a very potent factor. The local absorption, however, is sufficient for desirable therapeutic action, is the belief of the writer.

Following the requirements which a study of wounds suggests (Fauntleroy, Arthur), a preparation known as Paraffin "E" in this study was formulated, and given extensive therapeutic trials in various emergency clinics, as well as upon laboratory animals. The results were most gratifying. Under certain conditions paraffin preparations may be of doubtful value. Like all medical and surgical values it is capable of many contradictory results, and care in technique with intelligent observation has much to do with the results. The scientific preparation of the substance to be applied in the treatment is primarily essential.

The main considerations in the preparation of a substance of this character are as follows:

1. Aseptic cleansing of wound.
2. Drying and protection.
3. Flexibility of dressing.
4. Cohesion.
5. Adhesion.

If paraffin preparations are applied in an even, thin layer, forming a complete covering of the wound, the result will be as surely compatible with the requirements 3, 4, and 5 as though a heavy layer had been added. In fact, it is obvious that a number of layers applied, one superimposed upon the other, only tends to

stiffen the dressing, much as though a portion of the cake had been laid directly upon it without proper heating. The result would be only failure in some of the most desirable features of the treatment.

The value of paraffin as a dressing has only recently received its merited attention, and a study of cases in which it has been tried reveals several well-established *cures* which assume to a remarkable degree the appearance of fiction-like exaggerations. It is due to this that some highly colored and very impossible reports have been given wide-spread advertising and undesirable credence by those ignorant of the real conditions involved in surgical therapeutics. It should be remembered that a natural course of normal tissue repair must be brought about by encouraging growth metabolism under desirable and protective influences. This is the action of the various paraffins in greater or less degree, and these preparations have been tested severely for their mechanical and therapeutic values.

The formulæ tried in these experiments are briefly summarized in the following list, which covers fairly well the principal paraffins now to be found in certain well-known clinics:

Paraffin "A."

Resorcin, 1 per cent.
Eucalyptus oil, 2 per cent.
Olive oil, 5 per cent.
Paraffin molle, 25 per cent.
Paraffin durum, 67 per cent.

Paraffin "B."

Paraffin (51° C.), 70 grammes.
Liquid petrolatum, U. S. P., 3.0 Cc.
White beeswax, 10.0 grammes.
Resin, 7.0 grammes.
Resorcin, 0.2 gramme.
Sudan III, 0.05 gramme.
Alcohol, 10.0 Cc.

Paraffin "C."

Paraffin.
Resins.

Not analyzed, but found to be quite unstable in its constituency, the ingredients separating out upon heating, leaving the pure wax practically unadulterated by any substance whatever.

Paraffin "D."

Paraffin, 94-96 per cent.
 Gum elemi, 0.20-25 per cent.
 Japan wax, 0.40-50 per cent.
 Asphalt, 0.20-25 per cent.
 Eucalyptol, 2.0 per cent.

Color with 0.5 to 1.0 per cent solution alkannin in eucalyptol and minute quantity gentian violet.

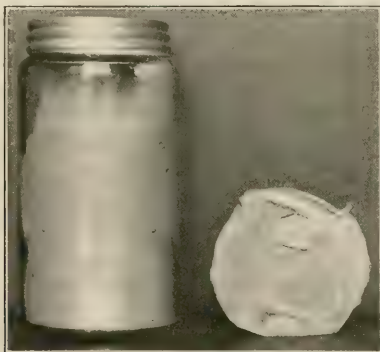
Paraffin "E."

Paraffin durum, about 75 parts.
 Paraffin molle, 25 parts.
 Olive oil, 5 per cent.
 High phenol coefficient oil; proportion (1:200).
 Chloretone, about 10 per cent.
 Colored with Scharlach R in alcohol.

Paraffin "F."—Same as "E," with solid extractives added.

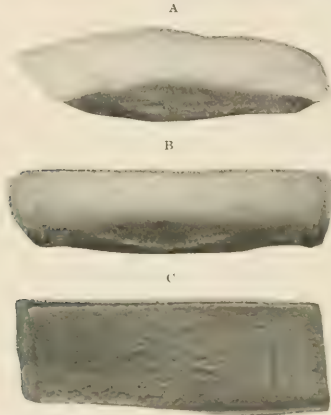
Paraffin "G."—Same as "F," with salicylic acid, about 3 per cent.

The greatest objections to paraffins A, B, C, and D seem to lie in the fact that they contain incompatible substances which precipitate as soon as the paraffin bases are melted so that they are freed. Preparations E and F were stable and homogeneous throughout all experimental trials. The case reports here given suggest proof of their varying values as therapeutic and surgical appliances.



Showing paraffin "F" after cooling in glass jar. The mass is perfectly homogeneous. No precipitation.

Case 1.—Male, thirty-one years of age. Accidental amputation of four fingers of right hand at phalangometacarpal articulations. Bones clean and protruding. Surrounding tissues receding, slightly lacerated. In order to make a flap it would



Sections showing comparison between perfectly suspended ingredients and precipitated ingredients in melted paraffins allowed to cool without stirring. "A"=paraffin B; "B"=paraffin D; "C"=paraffin E, without coloring matter.

have been necessary to amputate a portion of each metacarpal, destroying the otherwise complete palm. At the suggestion of the surgeon in charge the hand was dressed with paraffin "A" daily. No infection developed, and normal granulation with skin growth from edges of the wound showed complete healing with no noticeable scar in about four weeks.

It will be noted that skin-grafting was unnecessary, although a considerable portion of skin tissue was absolutely destroyed. Also no keloid developed.

Case 2.—Male, aged thirty-two. Indolent ulcer, about 5 or 6 square inches, following compound comminuted fracture of the tibia.

This case had resisted all treatment until a dressing of paraffin medicated with salicylic acid and hemostatic serum precipitates (Paraffin "G") was applied. The wound cleaned up with fresh

granulations in ten days, and one month from the date of initial application the ulcer had completely healed. This serum-paraffin was suggested by the marked action of serum upon similar ulcers as tried previously in hemostatic experiments.

Case 3.—Male, aged forty-four. Burn of second degree involving inner aspect of thighs and about pubic region from gasoline explosion.

This case was dressed with paraffin "F" daily, and discharged cured in three weeks. The skin developed from islets as expected, and scar tissue did not appear.

In the treatment of burns several statements recently made concerning the efficacy of paraffin preparations as being due solely to mechanical causes led the author to investigate this phase of the matter.

Samples of various formulæ were made up in the laboratory and stock market products were procured. The results are instructive and highly interesting. In this I had the coöperation of Dr. H. N. Torrey, Dr. Stilwell, and Dr. Dibble, of Detroit.

TABLE SHOWING CASE RESULTS SECURED WITH THE VARIOUS PARAFFIN PREPARATION FORMULÆ GIVEN ABOVE.

CASE.	WHEN SEEN.	TREATMENT.	RESULT.
Second degree burn on leg about 12 sq. in. circular margin.	Upon admission.	$\frac{1}{2}$ area with "A." $\frac{1}{2}$ area with "E."	"A" healed in 24 days. "E" healed in 21 days, no cicatrix.
Second degree burn from gasoline. About 40 sq. in., $2\frac{1}{2}$ in. average width.	Third day. Necrotic slough profuse.	$\frac{1}{2}$ area with "C." $\frac{1}{2}$ area with "F."	"C" showed clean strong granulations on 8th day, healed 32d day. "F" showed clean granulations 5th day, healed on 28th day.
Third degree lesion from corrosive poison injected by accident. A circular depressed wound about $\frac{1}{2}$ inch deep and 2 inches diameter.	On admission. Tissues non-purulent. Dead tissues sloughing.	Paraffin "F."	Daily dressings cleaned wound in 14 days. Healthy granulations and complete healing in 37 days.
Second degree burn, 6 inches wide; oval, 8 inches long.	Seventh day. Tissues infected slightly; suppuration established.	$\frac{1}{2}$ paraffin "B." $\frac{1}{2}$ paraffin "E."	"B" cleaned up in 5 days, healed 17 days later. "E" cleaned up in 4 days, healed 16 days later.
Second degree burn, about 26 sq. in.	Second day.	$\frac{1}{2}$ area with "D." $\frac{1}{2}$ area with "E."	"D" granulations clean and healthy 7th day, healed 29th day. "E" granulations clean and healthy 7th day, healed 27th day.

It will be seen from this table that in every instance the paraffin containing a high phenol coefficient tended to exhibit a constant superiority in germicidal action, and in the case of preparation "F" containing a certain suspension of serum derivatives the progress of the wound repair was markedly more rapid than with the other preparations.

It has been demonstrated above that the release of salicylic acid from properly balanced paraffin bases is brought about in sufficient quantities to have a very desirable inhibitive effect upon bacterial growth.

This is also seen by transplants from cultures incubated upon serum paraffin bases for twenty-four hours to which germicides were added.

It was mentioned above that many of the paraffin preparations are not made up with soluble materials, the ingredients settling out upon heating the paraffin base. This is not the case with preparations "E," "F," and "G," which remain a homogeneous mass when heated in the hot-water bath to the melting point. It was seen, however, that when melted over a flame the hot dish had a tendency to not only crystallize the paraffin, but coagulate certain ingredients and create a precipitation. It appears to the writer that if the material is used in the proper manner it will prove to be much superior to most of the substances now at the command of the surgeon, and many of the very plausible objections being offered will be found to be of very slight effect.

Of the method of application now in vogue nothing need be said except that in the experience of the writer and his associates cotton has been discarded and sterile gauze in a single thickness used between the first and second layers of the paraffin to give it "body." This does not stiffen so readily as cotton, and being more permeable gives a clean, even, and homogeneous mass to the dressing, which is not only easy to apply and remove, but very neat and attractive as well.

REFERENCES

- Beiter, J. R.: Observation of Paraffin Preparations, *Jour. A. M. A.*, June 16, 1917, p. 1801.
 Holl, A. I., Col. R. A. M. C.: Anchrine and its Substitutes, *Lancet*, Jan. 13, 1917.
 Howarth, E. B.: Speed Wax Preparation for the Treatment of Burns, *Jour. I. M. A.*, May 12, 1917, p. 1161.
 Lanthier, A. M.: Stages of Wound Infection During the Present War, *Trans. Am. Surg. Ass'n*, vol. xxxiv, p. 12.
 Arthur, W. H.: *Military Surgeon*, vol. xi, No. 5, May, 1917, pp. 189-92.

**Studies from the Research Laboratory.
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**REVIEW OF BLOOD COAGULATION STUDIES WITH
MENTION OF A NEW COAGULATION AGENT.**

BY VINCENT ANTHONY LAPENTA, M.D., AND GEORGE WALTERS, M.D.

[From the Research Laboratory, Parke, Davis & Company, Detroit, Michigan.]

Variations in the coagulating power of the blood are encountered in diverse manifestations of practical medicine. Excessive hemorrhages supervening upon a solution of continuity of the vascular walls, produced by traumatic or pathological processes, indicate diminished coagulability of the blood. Excessive transudation of lymph (serous hemorrhage) is intimately associated with this condition.

It also seems established beyond dispute that one of the most important factors of success and low mortality in modern surgery is the proper coagulation time of the blood. Indeed the most ideal conditions for an operation are intimately connected with the coagulability of the patient's blood. For these reasons a study of those agents which may enable one to attain this end is timely and interesting.

HISTORY AND DESCRIPTION.

In all cases of hemorrhage, the blood as it collects about the open wound should form an adhesion thrombus or coagulum by virtue of its inherent physiological properties, seal the openings in the vessels and prevent further flow. The formation of this coagulum depends upon the production of fibrin brought about by the action of fibrinogen and fibrin ferment (thrombin).

Thrombin is a product of blood coagulation. Fibrinogen and calcium are recognized constituents of the normal blood. In the process of coagulation thrombin is produced either from the combined action of basic elements in the blood or the near-by tissues.

Thrombin (Howell¹) is a derivative of prothrombin (proferment) which is associated with an antiferment which maintains.

under suitable physiological conditions, an ideal equilibrium. According to this theory a thromboplastic substance (*infra*) neutralizes the antithrombin normally in the circulating blood and thereby permits the interaction of calcium and prothrombin which initiates the coagulation process. In the presence of an excessive amount of antithrombin the reverse is true.

A general review of hemostatic therapeutics elicits many very striking differences of opinion both as to the cause and treatment of hemorrhage. This is due in great measure to the many diverse theories of the physiology of the circulation and the pathology of the blood constituents. Recent investigations have shed considerable light upon these theories. Variouslly stated, the coagulation of the blood is brought about according to the following illustrated table of consecutive chemical processes, which is based upon final results in the study of prothrombin (Howell, *loc. cit.*):

Thrombogen
(Leukocytes and
Platelets)

Thrombokinase
(Platelets and
tissue)

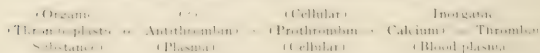
Thromboplastic substance
Neutralizing
Antithrombin

Prothrombin
combining
with Calcium

Thrombin
Fibrinogen

Fibrin
Clot

In fact, thrombin is shown by the following equation:



From this it will be seen that primarily the quantity of prothrombin or actual fibrin proferment present within the blood depends upon the properly balanced proportions of thromboplastic substance and antithrombin, an excess of the first increasing the prothrombin quotient by partial neutralization, or *vice versa*. Thromboplastin, or more properly thrombokinase (Howell¹), and antithrombin (Howell²), while easily demonstrated in the

blood serum, have the unsatisfactory relationship to practical biochemical products of purely hypothetical substances. For this reason, general interest in the biochemical features of blood coagulation as related to hemorrhagic diatheses has been limited to a few conscientious investigators who have attempted to arrive at some acceptable, non-contradictory evidence of an actual hemostatic or coagulating agent in the blood stream.

Starting with the first step in clot production following solution of continuity in the vessel wall from any cause, certain authors (Brodie,² Howell¹) presuppose a thromboplastic substance derived from thrombogen (nucleohistone) (Luciani²³) and thrombokinase. Thrombogen, it has been assumed, after various researches, is derived from the white cells, and possibly the platelets and the injured tissue cells, with which the blood comes in contact during active hemorrhage. The various theories are well summarized as follows (Drinker and Drinker⁴):

(a) Prothrombin arises from all the formed elements of the blood.

(b) Prothrombin arises from the leucocytes.

(c) Prothrombin arises from the platelets—(1) which are produced by leucocytes and erythrocytes; (2) which are independent elements.

(d) The formed elements of the blood have nothing to do with clotting.

(e) Prothrombin cannot be obtained from blood-free tissue, so its source of origin must be in the blood itself.

Howell,⁵ attempting to discover the source of prothrombin, was unable to determine positively its specific source. Lymph, bone-marrow (Drinker, *loc. cit.*, Duke,¹³ Nolf⁶); leucocytes (Morawitz,⁷ Bordet et Delange⁸); platelets (Wright,⁹ Morawitz,⁷ Nolf,⁶ Bayne-Jones,¹⁰ Deetjen,¹¹ Duke¹⁴); liver (Nolf¹²)—these are some of the many sources variously mentioned from which this substance is derived, and in each case some plausible explanations are submitted.

Assuming, however, that somewhere out of this maze the source of prothrombin may be definitely shown, we have another factor, and a somewhat more potent one, with which to contend. This is antithrombin, which may or may not vitiate the results

possible with the clot ferment. When this anti ferment is physiologically proportioned to the thromboplastic content, the resulting pro ferment, combined with a certain necessary physiologically balanced amount of calcium from the soluble lime salts normally in the blood stream, releases thrombin which, acting upon fibrinogen in the manner of a ferment or catalyzer, changes it to fibrin. **A clot is the ultimate result.**

Antithrombin has been shown to have a remarkably high counter-active potency against thromboplastic ferment (Howell³), and paradoxical as it may seem the thrombin ferments as a rule have a comparatively low potential when opposed to antithrombin.

It is therefore evident that in order to counteract the substance antithrombin, a more concentrated elemental factor resembling the kinase is required.

Much work bearing on the source of antithrombin has been done, with the result that many workers (Howell,³ Rich,¹⁶ and others) have demonstrated that the liver and intestinal glands are responsible to a great extent for its production. Rich,¹⁶ however, showed during experiments to determine the nature and properties of metathrombin that these glands are not entirely responsible, as complete occlusion of the vessels below the head blood supply did not stop antithrombin production in the head circulation. In view of this fact we must qualify the theory formerly advanced, at the same time accepting it in the main, since it has not been demonstrated that the major portion of the substance antithrombin is not generated, as shown by Howell.*

From the foregoing it will be seen that coagulation of the blood depends to no little extent upon the thrombokinetie coefficient of the serum. This is evidenced in the many cases of thrombosis in active febrile diseases where tissue destruction is pronounced (Mazentie¹⁷), and is also shown to be more powerful in autogenous than in heterogenous transplantations. This explains the many good results recorded of the use of fat and tissue transplants for hemostatic purposes (Risley,²⁶ Sacca,²⁷ Horsley,²⁸ and others).

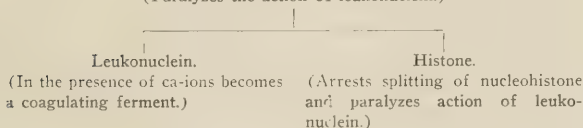
*The experiments on which the above are based have not many of the arguments upon which most bacteriologists base their conclusions as to the generation of bacteria.

Working on this theory, the senior author of this article has demonstrated a newly isolated blood derivative which may easily be looked upon as the product of the coagulation process in that it undoubtedly contains the specific thrombokinetic substance in nearly its pure form, in addition to the proferment prothrombin.

General Properties.—This new substance† is a clear light amber-colored solution containing the specific antagonist to anti-thrombin. In relatively minute quantities this substance has powerful specific action and represents ideally many of the requirements of a blood-coagulating agency. When properly protected from the action of heat it is perfectly clear. This makes it physically adapted to intravenous, subcutaneous, intraspinal, or intraperitoneal use. There is no inconvenient waiting while solution is being accomplished, and a possible source of contamination is thus removed. Physiologically its action brings up many features which tend to throw light upon certain of the coagulation processes. Before going into the details of the experimental work carried out, the authors wish to preface the work with the following scheme as worked out by Luciani,²⁵ which seems intelligently to sum up the preceding research conclusions explicitly and lay the way open for a definite working plan:

NUCLEOHISTONE.

(Paralyzes the action of leukonuclein.)



The predominance of one or other of these three substances gives rise to various normal or abnormal states of the blood which can be tested quite easily by injections of albumose or similar reagents.

The part which each takes in the coagulation process has been the subject of innumerable studies. Addis²⁸ and Morawitz²⁹ both show that the addition of calcium has been found not only *not* to

†The authors suggest the term Hemostatic Serum as the name by which this substance shall be known.

hasten coagulation but actually to delay it in many cases. As a rule physiological quantities of calcium are present, cases of calcipriva hemophilia being rare (Hess³⁰).

Coagulating Power.—Comparative observations on animals were made with standard hemostatic and blood coagulation agents, under parallel conditions. The anesthetics used were ether, chloroform, trichlorotertiarybutyl alcohol,* and the standard A. C. E. mixture. In most cases a preliminary hypodermic of morphine sulphate was given three-quarters of an hour before the time set for the anesthetic. The various results are shown in detail. The coagulation times were all taken with Bogg's modification of the Brodie-Russell coagulometer,³¹ and in many instances checked by the Biffi-Brooks instruments and test tube method of Howell.

Since the results with these methods showed no appreciable variations the report will be confined to estimations by the Brodie-Russell-Bogg instrument, which is deemed most reliable by the authors in that the various physical changes are constantly before the operator, and such technical differences as are effected by the amount of blood observed, intermediate coagulation process, temperature, etc., may be compared in the various specimens.

The wounds were made with a sharp pair of dissecting scissors on the anterior and lateral margins of the right lobe of the liver. Comparative observations were made with other well-known coagulating agents, separate wounds being cut for each observation. (Care was observed to wall off the previously injured area and clean the field before making new wounds.) Ether and chloroform anesthesia was employed in these operations as a rule; where any other anesthetic material was used it will be mentioned.

Protocol I.

The local hemostatic effect of the substance was tested by application on gauze to a resection of the liver approximately half an inch in depth. The degree of hemostasis is indicated by x, xx, and xxx. X indicates slight tendency to immobilize exudate which was pushed away by heavy blood flow; xx indi-

*Known commercially as chloretone.

cates hemorrhage held for half a minute or more; xxx indicates permanent clot formed. Products A and B at the end of one-half minute showed hemostasis of the x degree and after $1\frac{3}{4}$ minutes xx. The experimental serum derivative after half a minute showed xx, and after $1\frac{3}{4}$ minutes xxx.

The technique has much to do with the results obtained in this procedure. In applying the material the gauze should be applied directly to the wound and firmly held there for the interval of time desired, after which it should be carefully removed, avoiding any wiping effect by lateral motion, since this serves to brush away any formed thrombus. In order to ascertain whether any increased action might be expected from a preliminary injection the following experiment was tried:

Protocol II.

This was an experiment to determine the effect of local applications after preliminary injection of 1 Cc. of the substance 20 minutes before work was begun. The hemostatic effect of the various materials tested may be designated as follows:

Product A, after $\frac{1}{2}$ minute, x.

Product B, after $\frac{1}{2}$ minute, xx; after 1 minute, xxx.

Product C, after $\frac{1}{2}$ minute, xxx.

Experimental serum derivative, after $\frac{1}{2}$ minute, xxx.

Adrenalin, after $\frac{1}{2}$ minute, xxx.

Water (distilled), after $\frac{1}{2}$ minute, x; after 1 minute, x; after 2 minutes, xxx.

Anesthetic, chlorotone 0.2 gms. per kilogramme weight and A. C. E. mixture.

The normal coagulation time for this animal, a black adult bitch spaniel, about 11 kilogrammes, was 5 mins. 40 secs. Twenty minutes after the injection of 1 Cc. of the experimental material the coagulation time was 2 minutes 35 seconds, two separate tests being made. The observations covered a period of 2 hours 20 minutes, at the end of which the coagulation time was 2 minutes 40 seconds. Blood-pressure remained approximately normal, and the animal was killed. A rather surprising and unlooked-for action on the application of gauze soaked in distilled water brings out another feature in the coagulation process, probably due to

presence of minute quantities of alkali substances as well as the mechanical static effect upon the blood-flow when exerted long enough for it to coagulate and immobilize by virtue of the preliminary dose of the hemostatic agent.

This observation led to the following experiment to determine the actual effect on the coagulation time of the blood, *in vivo*, when the substance was employed:

Protocol III.

This experiment was carried out in order to determine the efficiency of the substance when given intravenously for the purpose of shortening the coagulation time of the blood. A maximum effect is sought. Large brown mongrel hound, about 16 kilogrammes. Apparently normal. No anesthetic.

On January 26, 1 mil (Cc.) of experimental serum derivative was given intravenously, coagulation at that time being found to be 6 minutes. Subsequent observations of the coagulation times on the same date showed them to be as follows:

3:05, 3 mins. 10 secs.; 3:10, 3 mins. 10 secs.; 3:40, 3 mins. 7 secs.; 4:40, 3 mins. 8 secs.; 5:15, 3 mins. 12 secs.

At 9 A.M. the following day the coagulation time was found to be 5 minutes 20 seconds, at which time another injection of 1 mil (Cc.) experimental serum derivative was given by intravenous route. Observations on the coagulation time following this injection were as follows:

9:20, 2 mins. 40 secs.; 10:20, 3 mins.; 11:30, 2 mins. 50 secs.; 2:30, 3 mins. 3 secs.

At 2:30 another injection, 2 mils (Cc.), of the experimental serum derivative was made intravenously. Coagulation times, following this injection, were as follows:

2:40, 2 mins. 30 secs.; 3:30, 2 mins. 25 secs.; 4:30, 2 mins. 35 secs.; 5:15, 2 mins. 30 secs.

At 9 A.M. the following day (Jan. 28, 1917) the coagulation time was 4 minutes. Dog was reinjected with 5 mils (Cc.) experimental serum derivative intravenously. At 9:20, coagulation time was 2 mins. 20 secs.; at 10:30, 2 mins. 30 secs.; 5:30, 3 mins. 25 secs. Two weeks later (2-10-17), coagulation time was tested, and found to be 5 minutes 50 seconds. At this time dog

was reinjected intravenously with 2 mls (Cc.) experimental serum derivative. Twenty minutes later, coagulation time was 3 mins. 5 secs.; 1 hour later, 3 mins.; and after 8 hours, 3 mins. 10 secs. No ill effects followed these injections, dog appearing to be healthy at end of treatment.

This animal was kept for two weeks more and finally used on other work. Blood specimens were taken from dry clean ear wounds, which produced a good drop in each case, the same instrument being used for all.

Repeated efforts to produce thrombosis with the new substance failed. In one instance 40 mls (Cc.) were given intravenously to a young adult rabbit weighing 1500 grammes, while 15 mls (Cc.) administered to a rabbit of 350 grammes did not produce any ill effect whatever.

Experiments on dogs also corroborate this work. The protocols here given demonstrate the results.

Protocol IV.

Small black mongrel, weight 11.25 kilogrammes; anesthetic chlorotone, 0.7 gramme per kilo.³³ Morphine $\frac{1}{2}$ grain hypodermic, $\frac{3}{4}$ hour before bringing to table. Anesthesia complete 1:15 P.M. Ten mls (Cc.) experimental serum derivative were given to this dog every hour until 5:15, a total of 50 mls (Cc.), and the next morning, the animal still being unconscious owing to the anesthetic used, another 30 mls (Cc.) were given intraperitoneally, making a total of 80 mls (Cc.) within 22 hours, or about $3\frac{1}{2}$ mls (Cc.) per pound of weight. During this observation the following coagulation times were recorded:

2/13/17, 1:15 P.M., coagulation time 5 minutes 5 seconds; 3:20 P.M., 2 mins. 30 secs.—dog had received at this time total of 20 mls (Cc.); 4:30 P.M., coagulation time, 2 mins. 28 secs.—total amount injected up to this time, 40 mls (Cc.); 5:30 P.M., coagulation time, 2 mins. 29 secs.—total amount injected, 50 mls (Cc.). 2/14/17, 9:30 A.M., coagulation time, 3 minutes; 10:40, coagulation time, 2 mins. 25 secs.—total amount which had been injected, 70 mls (Cc.); 12 M., coagulation time, 2 mins. 30 secs.—total amount given, 80 mls (Cc.).

Here again the fact of a minimum coagulation time for the blood (Rettger²²), when excessive doses of experimental blood derivative is given, indicates that with the stimulation of the hemopoietic and renal activities (*infra*) an increase in the antithrombin content of the blood takes place, proportionate to the amount of thrombokinetie factors added. (This theory is compatible with Luciani's scheme previously mentioned.) It is further elucidated by observation of the clotting power on sheep's plasma *in vitro*. The antithrombin in this case is, of course, constant for the experiment. In another portion of this paper the small percentage of total solids (1.43 per cent) and the specific gravity (1.0050) is commented upon. Since the calcium content of the serum is withdrawn in the preparation of this experimental blood derivative, this substance (calcium) in the form of the chloride was added for all tests *in vitro*, in physiological quantities, so that the desired results might compare with those of other coagulating agents. The method employed by the writer is easily followed in the table below.

Six test-tubes are used in this series with graduated quantities of the material as shown:

Degree of Coagulation.

Coagulating Substance.	Sheep Plasma.	Water, distilled.	Incubated 15 min. at 37°.	Incubated 25 min. at 37°.
.6 ml (Cc.)	.5 ml (Cc.)		xxx	xxx
.4 " (Cc.)	.5 " (Cc.)	.2 ml (Cc.)	xxx	xxx
.2 " (Cc.)	.5 " (Cc.)	.4 " (Cc.)	xxx	xxx
.1 " (Cc.)	.5 " (Cc.)	.5 " (Cc.)	xx	xxx
.05 " (Cc.)	.5 " (Cc.)	.55 " (Cc.)	x	xx
.025 " (Cc.)	.5 " (Cc.)	.575 " (Cc.)	x	x

xxx indicates solid coagulation throughout; xx, partial coagulation; x, slight coagulation (sticks to side, giving a curious "sucking" noise upon shaking free—no coagulation).

Upon adding 0.3 per cent of calcium chloride the whole set were coagulated with exception of highest dilution, which was xx. Upon adding 3 per cent calcium chloride, spontaneous coagulation took place, the tubes being solid before reaching the incubator.

An interesting comparative test made with Products A, B, C, and the experimental product above mentioned, is deemed worthy of mention in this paper.

The same dilutions as above were used. The results only are tabulated for 15- and 30-minute incubation periods:

Dilution.	Product A.		Product B.		Product C.		Exp. Serum Derivative.	
	15 min.	30	15 min.	30	15 min.	30	15 min.	30
No. 1	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
No. 2	xxx	xxx	xx	xxx	xxx	xxx	xxx	xxx
No. 3	xx	xx	xx	xx	xx	xxx	xxx	xxx
No. 4	—	x	xx	xx	xx	xx	xx	xxx
No. 5	—		—	x	x	xx	x	xx
No. 6			—	—x	—	—	x	xx

The superior clotting power of this serum derivative was evident throughout a long series of tests. Under anaphylactic reaction this agent is discussed again.

Physiological Action. Considering the low protein content, probably the most interesting feature of the physiological reaction of this substance is the effect it has upon the blood-pressure. This action has been carefully observed throughout the series of observations on animals and has been verified clinically by the results obtained in the operating-room. It was noted early in the clinical work that shock was a minor factor in postoperative treatment of those cases to which this substance had been administered. This was soon shown to be largely due to the effect upon the nephritic and hemopoietic system and the secondary effect upon the splanchnic nervous system. Observations were made with a standard mercury manometer, graduated, however, to percentage ratios rather than millimeters, it being at first intended only to show relative conditions of blood-pressure changes rather than actual pressure readings. The results are therefore slightly confusing to those not having observed the changes, but are at the same time obvious.

Crile¹⁹ demonstrated the effect of splanchnic nerve irritation to be widened principally in lowered blood-pressure. He also showed that any stimulation, crushing, mutilation, etc., generally showed slightly *increased* blood-pressure which remained so for a considerable period.²⁰ During the experiments with wounds of the liver (*supra*), it was noted that a general tendency to lowered blood-pressure was early presented, and that in most cases an intravenous injection of the derivative intended to increase

the blood-coagulating efficiency had the unexpected effect of increasing the pressure, restoring it to the normal or initial reading which was obtained immediately after perfect anesthesia had been produced.

Stimulated by these results, corroborative evidence was sought, which ultimately resulted in considerable study of the physiological action of the substance.

Stevens and Lee, 1884,²⁰ in the course of perfusion experiments, demonstrated the presence of an active vasoconstricting substance in defibrinated blood which was apparently due to some product of the coagulating process. O'Connor, 1911,²¹ verified these findings with the conclusion that epinephrin content was negligible as a determining factor. Falta and Flemming, 1911,²² confirmed these results.

Sollmann²³ showed that serum produces a direct vasodilator effect upon the isolated kidney, limited to the efferent vessels, and since then Brodie²⁴ demonstrated this action to be due to an albumen protein which in this substance is eliminated.

Before discussing its action the following protocols are of interest and serve to illustrate some of the facts to be mentioned:

Protocol V.

Well-nourished, healthy young black spaniel, weight 10 kilogrammes. Ether anesthesia. Immediately upon production of narcosis, a cannula was introduced in the left carotid artery and manometer attached. Normal reading showed at 50°—about the normal point for readings usually made with the type of manometer in use in any laboratory. All readings are comparative and the actual figures will be given in this protocol.

At 1:20 anesthesia was complete, blood pressure being 50 . At 1:24 intestines were handled roughly and exposed to air. At 1:50 blood-pressure was found to be 39 . At 1:52, 1 mil (Cc.) of the experimental serum derivative was injected subcutaneously. Following this observations of the blood-pressure were made as follows:

1:53, 45°; 1:54, 49; 2:25, 50; 2:45, 45; 3:30, 32; 3:45, 35°.

At 3:45 animal was reinjected subcutaneously with 1 mil (Cc.) experimental serum derivative.

3:47, blood-pressure of 43° ; 3:49, 48° ; 3:50, another subcutaneous injection of $\frac{1}{2}$ mil (Cc.) experimental serum derivative was made. At 4:05, blood-pressure of 53° , and at 4:20, 50° . Animal was killed at 4:20.

Duration of observation 3 hours and 20 minutes.

Pressures taken on peripheral and central circulations are also interesting.

Protocol VI.

Well-nourished bulldog, weight 16 kilogrammes; anesthetic, ether and chloretone.

Manometers connected with brachial and femoral arteries for peripheral pressures and portal vein, and renal artery for central circulation.

At 9:45 anesthesia was complete, blood-pressure being 51° ; 9:55, $\frac{3}{4}$ mil (Cc.) experimental serum derivative injected; 9:57, blood-pressure readings were as follows: Femoral, 56° ; portal, 58° ; renal, 55° .

At 10:15, $\frac{3}{4}$ mil (Cc.) was injected. Blood-pressure was found to be — femoral, 58° ; portal, 60° ; renal, 62° . At 10:30, femoral blood-pressure was 54° ; at 11:10, femoral 55° , portal 59° ; at 11:20, renal pressure, 61° .

Protocol VII.

At 11:20 the viscera were manipulated, and hemorrhage was produced in liver by making $\frac{1}{2}$ -inch resection.

At 11:35, 1 mil (Cc.) was injected, blood-pressure at that time being, femoral, 48° ; portal, 55° ; renal, 58° . At 11:40, pressure was femoral, 52° ; portal, 60° ; renal, 63° . At this time hemostasis was produced by tamponage with experimental serum derivative. At 12:15 pressure was femoral, 53° ; portal, 61° ; renal, 62° . At 2 P.M., femoral, 48° ; portal, 58° ; renal, 60° . At this time the condition of the animal was good; respiration was about 12 per minute. The viscera were exposed to cold and were freely manipulated. At 2:45, 1 mil (Cc.) was injected, the blood-pressure being femoral, 40° ; portal, 39° ; renal, 45° .

It will be observed that there had been a decided fall; the diastolic pressure was especially low.

At 3:05, the femoral pressure was 45; portal, 56; renal, 55. Pressure remained stationary until 4:15, at which time animal was killed.

Duration of observations, 6 hours and 30 minutes. All administrations were given intravenously. These observations were verified with other animals, slight difference in ratios being noted owing to varying physical conditions.

A gross interpretation of these results can only be made after careful consideration of all effects produced by this new substance. It will be remembered that thrombosis is practically an impossible result from its use; that there is no marked physiological reaction beyond the stimulation of central blood-pressures in a certain degree and the marked blood-coagulation effect.

Reviewing the work as a whole, it seems difficult to sum up all the facts which best serve to substantiate these views. In general, we may state that:

(a) The minimum coagulation time obtainable has been 2 minutes and 20 seconds.

(b) The blood-pressures at this time are normal.

(c) Under excessive shock producing operations, the use of the new blood derivative intravenously serves to maintain the normal blood pressure for hours. (Pressure readings indicate this to be due partly to stimulation of the adrenals.)

(d) Antithrombin is increased in the blood-stream nearly in proportion to the antagonistic elements present after a certain point has been reached. (See work on activity, *supra*.)

(e) Protein content is reduced to a minimum, as evidenced by the lack of anaphylactic shock over prolonged periods of observation.

Anaphylaxis.—Various experiments were tried on guinea-pigs and rabbits for anaphylactic reactions, but to date no serious alterations from the normal have been noted. This is in accordance with the fact that the percentage of total solids is only 1.45, and the protein content is very low. As related elsewhere in this paper Brodie¹ shows certain correlating facts concerning albumen proteins which in the case of the substance here mentioned are eliminated.

CONCLUSIONS.

In the light of the prevailing doctrines on blood coagulation, and the treatment of hemorrhage, it seems established that:

1. An ideal agent capable of increasing the coagulability of the blood and exhibiting high therapeutic value in hemophilia must contain in addition to prothrombin the specific thrombo-kinetic substances of the coagulation process.

2. The protein content of such product should be minimal, and the product must be active in small doses.

3. The administration of calcium salts in the treatment of hemorrhage is irrational because it is proven that an increase of ca-ions in the blood beyond the physiological point prevents coagulation, and that cases of diminished coagulability due to deficiency of calcium are rare.

4. A serum derivative based on the foregoing theories exhibits these true physiological requirements, giving uniform and quick results in all instances.

BIBLIOGRAPHY.

1. Howell, W. H.: *Am. Jour. Phys.*, vol. 29, 1911, p. 187.
2. Brodie, T. G.: *Essentials of Experimental Physiology*.
3. Howell, W. H.: *Also Arch. Int. Med.*, Jan., 1914, p. 76.
4. Drinker, O. K., and Drinker, K. R.: *Am. Jour. Phys.*, vol. xli, 1, July 1, 1916, p. 5.
5. Howell: *Am. Jour. Physiol.*, 1914, xxxv, 183.
6. Nolf: *Arch. Internat. d. Physiol.*, 1906, iv, 165.
7. Morawitz: *Deutsch Arch. f. Klin. Med.*, 1904, lxxix, 215.
8. Bordet et Delange: *Annals d l'Institut. Past.*, 1912, xxvi, 731.
9. Wright: *Jour. of Morphol.*, 1910, xxi, 463.
10. Bayne-Jones: *Am. Jour. of Physiol.*, 1912, xxx, 74.
11. Deetjen: *Zeitsch. fur Phys. Chem.*, 1909, lxxiii, 1.
12. Nolf: *Arch. Internat. d. Physiol.*, 1905, 6, iii, 1.
13. Duke: *Arch. Int. Med.*, 1913, xi, 100.
14. Duke: *Behav. of Platelets. Johns Hopkins Hosp. Bul.*, 1912, xxiii, p. 144.
15. Howell: *Human Physiology*.
16. Rich, A. R.: *Am. Jour. Physiol.*, 1917, xliii, 4, p. 549.
17. Mazentie: *Human Physiology*.
18. Crile: *Surg. Shock*, 1899, p. 130.
19. Crile: *Surg. Shock*, 1899, p. 132.
20. Stevens, L. T., and Lee, F. S.: *Studies Biol. Lab. Johns Hopkins Univ.*, 1884, p. 113.
21. O'Connor, J. M.: *Münch. Wchnschr.*, 1911, lviii, 1439.
22. Falta, W., and Flemming, G. B.: *München. Med. Wchnschr.*, 1911, lviii, 2649.
23. Sollmann, T.: *Am. Jour. Physiol.*, 1905, xiii, 291.
24. Brodie, T. G.: *Jour. Physiol.*, 1911-12, xliii, p. 321.
25. Luciani: *Human Physiol.*, 1911, vol. i, p. 138.
26. Risley, E. H.: *Surg. Gyn. and Obs.*, xxiv, 1917, p. 85.
27. Sacca: *Polyclinics*, Dec. 10, 1916.
28. Addis, T.: *Jour. Path. and Bact.*, xv, 1911, 427.
29. Morawitz, P.: *Handbuch d. Biochem. Arbeits methoden*, Bd. I, 1911, 233.
30. Hess, A. F.: *Johns Hopkins Hosp. Bull.*, vol. xxvi, 1915, p. 372.
31. Hinman, F., and Sladen, F. J.: *Johns Hopkins Hosp. Bull.*, vol. xviii, 1907, p. 213.
32. Rettger, L. G.: *Am. Jour. Physiol.*, 1909, xxiv, p. 434.
33. Rowe, L. W.: *Jour. Pharm. and Exp. Therapeutics*, 1916, vol. ix, p. 107.
34. Horsley, Sir Victor: *Br. Med. Jour.*, July 4, 1914, p. 8.

Studies from the Research Laboratory.

Parke, Davis & Co.

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PEPTONE-FREE MEDIA FOR ROUTINE CULTURE WORK.*

BY N. S. FERRY, M.D., AND ARLYLE NOBLE, A.B., DETROIT, MICH.

As a result of some experiments which necessitated the use of culture media prepared without peptone, it was found that the organisms under observation grew as luxuriantly as on standard media, suggesting the possibility of employing such media for routine purposes. It was determined, therefore, to give the method a comprehensive trial, as it involved a question of practical nature, especially to laboratories where large quantities of media are consumed daily.

Ten different kinds of media were prepared without peptone, part of them neutralized and part made 1.0 per cent acid, phenolphthalein being used as the indicator.

- | | | | |
|-----|---------------|----------|---------------------------|
| 1. | Liebig's Ext. | bouillon | —without peptone—neutral. |
| 2. | " | " | " " —1.0% acid. |
| 3. | Beef | bouillon | " " —neutral. |
| 4. | " | " | " " —1.0% acid. |
| 5. | " | agar | " " —neutral. |
| 6. | " | " | " " —1.0% acid. |
| 7. | Veal | bouillon | " " —neutral. |
| 8. | " | " | " " —1.0% acid. |
| 9. | " | agar | " " —neutral. |
| 10. | " | " | " " —1.0% acid. |

A large variety of organisms was grown on the above media, culturing for several generations both freshly isolated strains and strains of various ages. For comparison, the same strains were grown on the standard media with Witte's peptone.

A tabulation of some of these results is given.

The figures in the charts are to be interpreted as follows:

*From the Research Department, Parke, Davis & Co., Detroit, Mich.

1+ = Better than normal (on control media).

1 = Normal, abundant growth; first choice (except for an occasional 1+).

1- = Abundant growth, but not quite normal; second choice.

2+ = Abundant growth; third choice.

2 and 2- = Moderate growth.

3 = Poor growth.

4 = Very slight growth.

GROWTH IN BOUILLON

ORGANISMS	MEDIA WITH WITTE'S PEPTONE					MEDIA WITHOUT PEPTONE														
	Plain Bouillon			Liebig's Ext Bouillon		Veal Bouillon						Beef Bouillon								
	1.0% Acid			Neutral	0% Acid	Neutral			1.0% Acid			Neutral			1.0% Acid					
	1st gen	2nd	4th	1st gen	1st	1st gen	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th			
Staphylococcus aureus	1	1	1	3	4	1	1	1	2+	1	1	1	1	1	1	1	1	2+	1	1
Staphylococcus citreus	1	1	1	3	4	1	1	1	2+	2+	2+	1	1	1	2	1	1	2+	1	2+
Staphylococcus albus	1	1	3	2+	-	1	2+	1	1	1	1	1	2	1	1	1	1	2	1	2
B. pyocyaneus	1	1	1	2+	-	1	2+	1	1	1	1	1	2	1	1	1	1	2	1	2
B. coli	1	1	1	3	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B. paracoli	1	1	1	3	4	1	1	1	2+	1	1	1	2	1	1	1	1	2+	2	2
B. typhosus	1	1	1	3	4	1	1	1	2+	2+	2+	1	1	1	1	1	1	2+	2	2
B. paratyphosus A	1	1	1	3	4	1	1	1	2+	2+	2+	1	1	1	1	1	1	2+	2	2
B. paratyphosus B	1	1	1	3	4	1	1	1	2+	1	2+	1	1	1	1	1	1	2+	1	2
Streptococcus	1	1	1	4	4	1	1	1	2+	1	1	1	2	1	1	1	1	2	1	1

1st generation planted from 24-hour growths on agar into the above bouillons

2nd transplanted from the 24-hour bouillon growths

4th transplanted from third generation after being grown 24 hours and then placed in ice chest for 18 hours

GROWTH ON AGAR

ORGANISMS	MEDIA WITH WITTE'S PEPTONE				MEDIA WITHOUT PEPTONE											
	Plain Agar				Veal Agar						Beef Agar					
	1.0% Acid				Neutral						1.0% Acid					
	1st gen	2nd	4th		1st	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th
<i>Staphylococcus aureus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1-	1	1	1-
<i>Staphylococcus citreus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Staphylococcus albus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1-
<i>B. pyocyaneus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. coli</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. paracoli</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. typhosus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. paratyphosus A</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1-
<i>B. paratyphosus B</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Streptococcus</i>	Acetic Agar				1	1	1	3	2+	1-	2+	1	1-	1-	2+	1-
<i>B. diphtheriae</i>	Loeffler's Blood Serum				1	1	1	2+	2	2+	1-	1	2+	1-	1-	2+

1st generation transplanted from 24-hour growths on plain, acetic and Loeffler's Blood serum agar.

2nd generation transplanted from first after 24 hours.

4th generation transplanted from third after being grown 24 hours and then placed in ice chest for 6 days

When two or more culture media are represented by the same figure, it indicates that there was no choice between them.

All results were read at the end of twenty-four hours. With each organism several generations were watched, not only in the incubator, but also at 5° C., so that the conclusions were not arrived at after a single generation at 37° C.

CONCLUSIONS.

1. On peptone-free agar all cultures which are ordinarily grown on standard plain agar did so well that it was difficult to choose.

2. For the majority of cultures, veal broth media gave better results than beef broth, both with and without agar.

3. Both veal and beef broth gave far better results than Liebig's beef extract.

4. Throughout the whole experiment the neutral media gave better results than the 1.0 per cent acid.

5. Therefore, for organisms which grow readily on standard plain agar, neutral veal or beef media, without peptone, can be substituted.

**Studies from the Research Laboratory.
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**SERUM VEAL AGAR: A DEPENDABLE SUBSTITUTE
FOR ASCITIC OR BLOOD AGAR.***

BY N. S. FERRY, M.D., AND ARLYLE NOBLE, A.B., DETROIT, MICH.

For reasons obvious to those interested in the routine culture of a large number of organisms, and especially mass growths, it was necessary to find, if possible, a substitute for ascitic or blood agar. Consequently a large number of different media were tested and it was determined that a veal agar (neutral to phenolphthalein) to which had been added normal horse serum (or any other serum) would successfully accomplish the purpose.

It was found, also, that the organisms would grow, in most instances, as well, if the peptone were omitted from the formula. This is an exceedingly practical point, especially where large quantities of peptone are consumed. (See "Peptone-free Media for Routine Culture Work," page 298 of this issue.)

FORMULA FOR SERUM VEAL AGAR.

1. To 500 grams of lean chopped veal add 1,000 c.c. water. Macerate and allow to stand in refrigerator 24 hours. Strain through cheesecloth and bring to boil.

2. Filter, add 20 grams peptone (this may be omitted), 5 grams NaCl, and 30 grams finely chopped agar-agar.

3. Boil and adjust reaction to the neutral point with phenolphthalein (the success of the results with the medium depends in a large measure upon this point).

4. Filter and pour into test tubes, 3 c.c. each.

5. Sterilize fractionally, cool to about 45° C. and add 2 c.c. of sterile normal horse serum to each tube. The preparation should be about three parts of agar to two parts of serum.

*From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.

		GROWTH ON														
		WHOLE BLOOD AGAR					ASCITIC AGAR					SERUM VEAL AGAR				
Number of genera- tion after experi- ment starts		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of days in ice chest between transplants.....		4	4	6	14	17	4	4	6	14	17	4	4	6	14	17
Pneumococcus																
No. Type																
3 -I.		1		1	1	1	3	2	3	-		2	2	1	1	2
4 III		1	1	1	1	1	3	2	1	-		1	1	1	1	1
5 I.		1	1	1	1	1	2	1	1	3	2	1	1	1	2	1
6 II.		1	1	1	1	1	3	1	2	2	2	1	1	1	1	1
7 III		1	1	1	1	1	2	2	2	2	2	1	1	1	1	1
8 IV.		1	1	1	1	1	3	1	2	1	1	1	1	1	1	1
9 III.		1	1	1	1	1	3	1	1	-		1	1	1	1	1
10 I.		1	1	2	-		3	3	3	-		3	2	1	2	2
11 II		1	1	1	3	2	3	3	2	-		2	1	1	2	2
12 III		1	1	1	1	1	2	1	2	1	3	1	1	1	1	1
13 I.....		1	1	1	2	1	3	3	3	-		2	1	1	1	2
15 II.....		1	1	1	1	1	2	2	1	3	2	1	1	1	1	1
16 I.		1	1	1	3	3	3	2	2	-		2	1	1	1	2
17 I.		1	1	1	-		3	3	3	-		3	2	1	2	2
18 I.		1	1	3	-		3	3	2	-		3	2	1	2	2
19 I..		1	1	3	-		3	3	3	-		3	2	1	2	2

		GROWTH ON									
		ASCITIC AGAR					SERUM VEAL AGAR				
Number of generation after experiment starts.....		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of days in ice chest between trans- plants		6	6	14	14	14	6	6	14	14	21
Streptococcus from tonsils											
No. 1 (a)		1	1	1	1	1	1	2	2	1	1
1 (b).....		1	1	1	1	1	1	1	1	1	2
3 (a)		1	1	1	1	1	1	1	1	1	1
3 (b)		1	1	1	2	1	1	1	1	1	1
13		1	1	1	2	1	1	1	1	1	1+
14		1	1	1	1	1	1	1	1	1	1+
15		1	1	1	1	1	1	1	1	1+	1+
22..		1	1	1	1	1	1	2	1	1	1
33		1	1	1	1	1	1	1	1	1	1
43		1	2	1	2	1	3	2	1	3	1
45		1	1	1	1	1	1	2	1	2	1
56 (a)		1	1	1	1	1	1	2	1	2	1

	GROWTH ON														
	WHOLE BLOOD AGAR					ASCITIC AGAR					SERUM YEAL AGAR				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of generation after experiment starts	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of days in ice chest between transplants.	14	6	14	14	14 21	14	6	14	14	14 21	14	6	14	14	14 21
<i>Streptococcus viridans</i>															
No. 1	1	1	1	1	1	1	2	1	1	3	1	1	1	1	1
2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
3	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1
4	1	1	1*	1	2	3	1	3	-	-	3	1	1	1	1-
5	1	1	1	1	1	1	1	2	1	1-	1	1	1	1	1
6	1	1	1	1	1	2	2	2	2	3	1	1	1	1	3
7	1	1	1	1	1	1	2	2	1	3	1	1	1	1	1-
8	1	1	1	1	1	1	3	1	-	-	1	1	1	1	-
9	1	1	2	1	3	2	3	2	-	-	1	1	1	1	1-
10	1	1	1	1	1	2	3	3	2	-	1	2	1	1	3
11	1	1	1	1	3	1	2	3	1	-	1	1	3	1	3
12	1	1	1	1	2	2	1	2	2	-	2	1	1	1	3
<i>Streptococcus hemolyticus</i>															
No. 1	1	1	1	1	1	1	1	1	1	1	2	1	1	1+	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	2	2	2	1	1
6	1	1	1	1	1	1	1	1	1	1	2	2	2	1	2
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	2	2	2	1-	1
10	1	1	1	1	1	1	1	2	1	1-	1	1	1	1	1
11	2	1	1	2	3	3	2	3	-	-	2	1	1	-	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

ORGANISMS TESTED.

1. *Diplococcus pneumoniae*, Types I, II and III, cultures of which had been kept on whole blood (rabbit) agar. The majority of these cultures had recently been isolated.

2. *Streptococcus viridans* and *hemolyticus*, cultures of which had been kept on whole blood agar.

3. Various strains of streptococci which had been cultured on ascitic agar. The majority of these were fresh cultures from tonsils.

The following charts will convey some idea of the results of the tests. The figures in the charts are to be interpreted as follows:

- 1 or 1+ = Normal growth and is to be considered first choice.
- 1- = Abundant growth, but not quite as good as on control media.
- 2 = Moderate growth.
- 3 = Poor or slight growth.

All readings were made after 24 hours at 37.5° C. The cultures were then placed in the refrigerator, as has been the routine custom for ascitic or blood agar cultures, for the time indicated on the charts, ranging from four to seventeen days.

CONCLUSIONS.

1. Cultures of *diplococcus pneumoniae* grow as well on serum veal agar, after the second generation, as on whole blood agar and far better than on ascitic agar. These cultures lived longer on serum veal agar at refrigerator temperature, without transplanting, than on whole blood agar or ascitic agar. After two weeks four out of sixteen cultures on whole blood agar and ten out of sixteen on ascitic agar were dead, while all on serum veal agar were alive after seventeen days, and grew well when transplanted on the same media.

2. Cultures of streptococci grew as well and lived as long on serum veal agar as on whole blood agar and better than on ascitic agar.

3. Serum veal agar (veal agar, 2 per cent peptone, neutral, plus normal horse serum) can be used for maintaining cultures of most organisms ordinarily kept on ascitic or whole blood agar.

Studies from the Research Laboratory.

Parke, Davis & Co.

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A DISCUSSION OF SOME PRINCIPLES OF ANTHELMINTIC MEDICATION.

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There are certain general principles of anthelmintic medication that may be more or less tentatively proposed from the standpoint of theory, or from the clinical experience of many workers, or on the basis of experiments performed in this laboratory up to the present time. Some of these principles seem to me to be fairly well established; others are in contradiction to common beliefs and so open to more suspicion of error. At this stage of our knowledge in regard to anthelmintics, statements should be made tentatively, as a rule. It is a subject which needs and deserves much more study.

The general principles which, for the most part, I wish to discuss, rather than to urge, are as follows:

1. Anthelmintics are selective in their action; at least their dependable effectiveness is limited to certain sorts of worms.

This follows from differences in structure, physiology and habit on the part of the worms. This principle would scarcely seem worth stating or defending, except for the fact that some writers have disputed it. Most medical practice assumes that it is true, and numerous experiments in this laboratory show that certain drugs which display great anthelmintic properties against certain kinds of worms may be given in doses approximating the lethal dose or given in therapeutic doses over long periods of time without exerting any apparent effect on other worms occupying similar positions in the digestive tract. These experiments, which will be published later in the discussions of anthelmintics, confirm the general idea that certain drugs are of value against tapeworms, but not against nematodes; that others are of value against ascarids, but not against hookworms; that others are of value against hookworms, but not against tapeworms; etc. It is true.

to be sure, that such a drug as male-fern will occasionally remove some hookworms, and that such a drug as oil of chenopodium will remove an occasional tapeworm, but so far as dependable action is concerned, anthelmintics cannot be used over such a wide range.

Along this same line, it may be said that anthelmintic activity on the part of a drug is not proportional to antiseptic properties and that the published statement to the effect that verminous infestations can be combated best by antiseptic treatment cannot be accepted. Some of the best anthelmintics have very little germicidal power, while experiments show that excellent germicides have little anthelmintic power and that even those displaying a moderate anthelmintic power are usually severe in their effects on the host animal. In passing, it may be noted that germicidal power and insecticidal power are likewise unrelated as a rule.

2. As a further development of the above principle of anthelmintic selectivity, it may be stated that certain kinds of worms require not only a suitable anthelmintic, but also suitable modes of medication, whereas other worms only require the simple administration of a single therapeutic dose of a suitable drug.

The above statement may be briefly elaborated as follows:

(a). Forms like the ascarids, which inhabit the small intestine and lie unattached in the lumen, are readily accessible to the ordinary types of anthelmintics—the type which is usually said to be comparatively insoluble in the host intestine, and so believed to exert its effect on the worms with which it comes in contact while producing a minimum amount of systemic effect on the host owing to low solubility and slight absorption—and ascarids may be entirely removed by a single therapeutic dose of oil of chenopodium in the great majority of cases if only the usual precautions as to preliminary fasting are observed.

In this connection it may be noted that Lutz¹ states that ascarids, probably on account of their size, are more difficult to remove than other worms. All our experience is distinctly contrary to this, ascarids being the easiest of all worms to remove. However, Lutz was referring especially to the necessity for repeated treatments with santonin, and all our experiments confirm the idea that repeated doses of santonin are usually necessary to assure the removal of ascarids. Lutz did not have a

suitable drug for the removal of all ascarids at a single dose. As a matter of fact, the action of oil of chenopodium against ascarids in removing 100 per cent of all worms present in a large majority of cases, where it has been properly used in single dose in the experiments in this laboratory, is not duplicated by the action of any other drug in single dose against any worm in experiments here or in the work of Hall and Foster² at Washington.

Contrary to Lutz's statement, Railliet³ states it as a general rule that large worms are more vulnerable than small ones. This accords with our experience: Adult ascarids are more easily removed than larval ascarids or the smaller hookworms; hookworms are more easily removed than *Strongyloides* or the small trichostrongyles, the last two being very difficult to remove, so much so that Stiles⁴ has recently stated that no satisfactory treatment for *Strongyloides* in man is known. This condition may arise in part from the ability of small worms to protect themselves by a position in small food masses or under mucus.

(b). Forms like the hookworm, which attach for indefinite periods to the intestinal mucosa and occasionally detach and remain free in the lumen of the intestine for a time before attaching again, show a response to anthelmintics which may perhaps be correlated with this habit of attaching and detaching. In experiments where there is more than a very light infestation it appears that one anthelmintic treatment will usually remove only a part of the worms, and the same is true in clinical experience. As Lane⁵ says: "No single course of treatment by any known drug or combination of drugs can be relied upon to expel all the ankylostomes which are present." It is possible that a particular anthelmintic is effective only or principally against those worms which are attached at the time, or only or principally against those which are detached at the time, as the case may be. And it is possible that one anthelmintic would be effective against those which were attached while another might be effective against those which were detached. If this is true, effective treatment will commonly require repeated doses of anthelmintics in order ultimately to remove all the worms present, or it will require a combination, as yet unknown, of remedies that will exert a complementary effective action, *i.e.*, one acting on the attached and one on the detached worms. Of course, this ex-

planation of the failure of single hookworm treatments to achieve more than partial success, as due to the presence of one attached and one detached group of worms, is only theoretical. Other explanations would be that worms which were feeding at the time of treatment were susceptible to the anthelmintic, that individual worms had greater resistance or were better protected by mucus, etc. There is little evidence one way or another. In support of the idea that successful treatment is related to the attaching habit, there may be cited the suggestion noted by Hall and Foster⁶ that the efficacy of chloroform against hookworms may be due to the rapid absorption of the chloroform by the blood and the ingestion of this chloroform-containing blood by the attached hookworms in amounts sufficient to produce anesthesia or lethal toxic effects.

(c). Forms like the whipworm, located for the most part in the cecum, are apparently not always exposed to the action of anthelmintics even when adequate doses of potent drugs are given. This is not merely due to the fact that anthelmintics are partly absorbed and more or less diluted by the time they reach the ileocecal valve, though this factor must be taken into consideration in anthelmintic treatment for parasites located in the large intestine, but it is principally due to the fact that not everything that passes the ileocecal valve enters the cecum. This is a point that I have previously noted⁷ and in this connection I have suggested that in treatment of whipworm cases it may be found good practice to administer anthelmintics in small doses over a long period of time to ensure the entrance of the drug into the cecum. The experiment work done in the laboratories of the U. S. Bureau of Animal Industry and in this laboratory indicates definitely that a number of anthelmintics are effective against whipworms when they come in contact with the worms. As a rule the administration of an anthelmintic brings away no worms, but when it brings away any, as it occasionally does, it not uncommonly brings away all of them. Experiment work done in this laboratory indicates that the administration of small doses— which must not, however, be too small— of anthelmintics over long periods of time, in order to ensure their entrance into the cecum, is apparently the best mode of treatment for these worms. It is necessary to work out for each drug the minimum dose which exerts any anthelmintic efficacy, as it appears from experiments that too small doses will

have no effect even if administered over very long periods. Fortunately, the whipworm appears to have comparatively little resistance to drugs that actually reach it. This is the more surprising in view of the fact that the whipworm has the habit of sewing the long narrow anterior portion of its body into the mucosa. It may be that the exposed posterior portion is the part through which the anthelmintic takes effect, or it may be that the anthelmintic is absorbed by the cecal mucosa and takes effect, perhaps by ingestion, on the part buried in the mucosa.

In man, the presence of the appendix constitutes another complication. Presumably only a part of the intestinal contents which pass the ileocecal valve enters the cecum, and of this only a part in turn enters the appendix. In other words, it would probably take a certain amount of repeated treatment to remove worms from the cecum and an even greater amount of treatment, as a rule, to remove them from the appendix. Berard and Vignard⁸ state that it has not been proved that anthelmintics exert any effect on parasites in the appendix, and that Railliet's clinical experiments seem to show that the worms remain alive after the administration for several days of the most powerful vermifuges. Berard and Vignard reach the rather surprising conclusion that it is necessary to resect the appendix as a step in eliminating intestinal worms.

(d). Just in passing, it may be noted that in the field of veterinary medicine the administration of anthelmintics is complicated by anatomical considerations in the case of the host animal which are not present in the case of man. Thus the work of Hall and Foster in the Bureau of Animal Industry showed that in a general way volatile anthelmintics, such as chloroform, should not be administered to ruminants, not only because of the incidental danger of inhalation pneumonia, but also for the reason that such substances go very largely to the rumen, or storage stomach, and are absorbed with resultant systemic effects before they can pass to the abomasum, or true stomach, to exert anthelmintic action. This finding has recently been published in a note by Dr. Ransom, the chief of the Zoological Division of the Bureau. It is probable that the substances named are largely volatilized by the time they have passed through the esophagus.

3. Fluid extracts or other preparations using alcohol as a

solvent for active anthelmintic ingredients are frequently unsuitable as anthelmintics.

Experiments in this laboratory on a number of such preparations indicate that there are good objections to some of these preparations. In the first place, the very fact that alcoholic preparations are adapted to the production of rapid systemic effects—the effects one wishes to avoid in using the characteristically toxic group of drugs known as anthelmintics—is one reason why they are unsuitable as anthelmintics. These alcoholic preparations are often rapidly absorbed, largely in the stomach and duodenum, occasioning more or less irritation at the point of absorption and producing systemic effects of a more or less toxic nature. The considerable and rapid absorption leaves a comparatively small amount of drug available for actual anthelmintic action, and by the same token leaves the minimum of drug that could possibly be removed by purgation after exerting its anthelmintic effect. In the writer's opinion some alcoholic preparations of anthelmintics are distinctly dangerous to the host animal and relatively ineffective against parasites, and this opinion is substantiated by quite a number of experiments in this laboratory and seems to accord with the record of a number of "worm remedies" of this character.

This opinion is rather contrary to that expressed by Eeckhout. He makes one group of anthelmintics for those which are toxic and highly soluble and in which the absorbed material is rapidly eliminated. The important members of this group he names as follows: Turpentine, carbon bisulphide, chloroform, ether and alcohol. According to him, turpentine and carbon bisulphide are very toxic for intestinal worms, while chloroform, ether and alcohol are much less so, but also less dangerous to the host. He states that it is always an advantage to incorporate in a vermifuge some such narcotic as chloroform, ether or alcohol. In the writer's experience, turpentine is distinctly anthelmintic; carbon bisulphide is apparently anthelmintic but needs further study to determine its indications; chloroform has decided anthelmintic value against hookworms, but comparatively little against ascarids, whipworms, etc.; ether has very little anthelmintic value; and alcohol has practically none. If alcohol had anthelmintic value, it would appear that the human race should have been freed from most of

its parasites long ago. Moreover, as stated above, experiment shows that alcohol is distinctly disadvantageous in some anthelmintics; it makes some active principles soluble, but while the absorbed alcohol solvent is rapidly eliminated, the active anthelmintic ingredients simultaneously absorbed are not rapidly eliminated but are left to injure the host animal. If alcoholic preparations of anthelmintics are to be used at all, it would seem advisable to dilute them very greatly before administration in order to throw the anthelmintic out of solution and delay absorption. In some anthelmintics it is probable that the active constituents would be promptly thrown out of solution on contact with the buccal, esophageal and gastric mucosa, and that there would be little or no rapid absorption.

It is interesting to note that Bozzolo⁹, who was the first to use thymol against hookworm, in 1879, originally advocated the administration of a glass of strong wine or some alcoholic mixture after each dose of thymol in capsules, in order to facilitate solution of the drug. Thirty years later, he is still of the opinion that his patients were not poisoned and thinks the thymol was probably more effective when given in this way.

4. Anthelmintics of the supposedly insoluble type are not as insoluble as they are commonly supposed to be.

This fact was shown in the investigations of thymol by Schultz and Seidell¹⁰ and by Seidell¹¹. Seidell states: "Of the thymol administered, from one-half to two-thirds is apparently destroyed or temporarily fixed in the body." "The simultaneous administration of olive oil apparently caused very slight if any effect upon the percentage of excreted thymol. It is a question, therefore, whether oils really increase the amount of absorption of thymol or only the rate."

The writer's experience bears out what is said and suggested above. In giving large or repeated doses of oil of chenopodium, the feces commonly have the distinctive odor of this oil, but small doses do not give this result as a rule, and must be very largely absorbed. The elimination of this drug or its fate after absorption does not appear to have received sufficient study as yet. Salant and Livingston¹² state that after intravenous injections of large amounts of oil of chenopodium it may be detected in the respired air, but not in the urine or bile. However, I have been

unable to find any papers dealing with tests for oil of chenopodium in the urine or stating the form or product in which this oil might be expected to occur after absorption and modification in the body.

There is quite a little written in the literature of anthelmintics in the way of warning regarding the use of such solvents as castor oil, milk, alcohol, etc., in connection with the use of certain drugs, such as thymol and oleoresin of male-fern. It is commonly stated that as these drugs are soluble in the solvents specified, the use of these solvents will result in the absorption of an otherwise comparatively insoluble drug. It appears from the work of Schultz and Seidell and of Seidell that these comparatively insoluble drugs are largely absorbed in any case, regardless of the simultaneous use of such solvents as olive oil. Seidell makes the suggestion, noted above, that oils increase the rate of absorption rather than the amount. This seems to be a very good point and one that deserves some elaboration. In my opinion, there are two factors to consider in connection with the rate of absorption. One is the local effect of rapid absorption and the other is the systemic effect. When such toxic drugs as anthelmintics are rapidly absorbed, it means that the brunt of the irritation and insult due to the drug is borne by a rather limited section of the digestive tract; that a large amount of comparatively concentrated drug is taken in over the gastric and duodenal mucosa, and possibly by that of the upper jejunum. Experiments, clinical symptoms, and the occasional necropsy on the human victim of an overdose of anthelmintic show that such drugs as male-fern, thymol or oil of chenopodium can be highly irritating to the digestive tract when absorption is sufficiently rapid. In experiments in this laboratory, the post-mortem examination after the ingestion of these and similar drugs not infrequently shows an unsuspected degree of gastroenteritis and suggests that this condition is present in the human patient rather often, an idea which finds support in many published reports. In addition to the local irritation of the digestive tract by rapidly absorbed anthelmintics, there is the factor of the systemic effects due to the sudden imposition of a considerable amount of anthelmintic which must be disposed of by the body economy in bulk instead of piecemeal. It would appear that what is desired in anthelmintic administration is, first,

as little absorption as possible, and second, since there must be some absorption, probably a large amount as a rule, it is desirable to retard that absorption in order to distribute it over as large a surface of the gastro-intestinal mucosa as possible and to allow the maximum time for the body gradually to dispose of the drug by oxidation and elimination.

In this connection I wish to note that in my experiments olive oil seemed to be decidedly contraindicated in connection with anthelmintics, whereas I did not find this to be the case with castor oil. Salant and Nelson¹³ state that oils and fats, including olive oil, probably reduce the toxicity of oil of chenopodium, though they did not find this action constant. It is possible that they are right in attributing such a result to the modifying action of glycerides, as well as to the suppression of acute symptoms by the oils, and yet other factors may offset this action. In the case of olive oil, our post-mortem examinations indicate that mixtures of this oil and oil of chenopodium are absorbed largely in the stomach of experiment dogs with the production of gastritis, sometimes very severe. In this connection we may note the following statements in regard to olive oil by Asnis¹⁴: "Because hydrochloric acid has no effect on the olive oil and because of all foods it remains longest in the stomach, hence as a lubricant and as a protective agent it is unexcelled." Chenopodium itself exerts a depressant effect on the musculature, tending to produce stasis. In our experiments, the tendency of a mixture of olive oil and oil of chenopodium is to remain in the stomach until largely absorbed, causing gastritis, and this constitutes an objection to the combination. It is undesirable that an anthelmintic intended to remove worms from the small intestine should remain in the stomach producing irritation and undergoing absorption. In other words, the action of the olive oil is very similar in this respect to that of alcohol.

It is the purgative character of castor oil, its tendency to move promptly through the digestive tract, which appears to be responsible for the more satisfactory results obtained from the combination of castor oil and oil of chenopodium. Although there is absorption of castor oil as well as of olive oil, the absorption of castor oil appears to be such as to distribute the chenopodium content over a wider area and a longer period of time.

As regards the common statement that castor oil should not be given in connection with the oleoresin of male-fern, I hesitate to question what is evidently intended as a safeguard in the administration of this drug. However, I wish to state that I have given oleoresin of male-fern in doses of 20 mls—a dose Winslow¹⁵ mentions as lethal—to a number of dogs as follows: Dog No. 145, weighing 11.5 kilos, received 20 mls of oleoresin of male-fern and 60 mls of castor oil; the dog survived the treatment and was killed 6 days later. Dog No. 131, weighing 7.5 kilos, received 20 mls of oleoresin of male-fern and 60 mls of castor oil; the dog survived the treatment and was killed 8 days later. Dog No. 171, weighing 8.25 kilos, received 20 mls of oleoresin of male-fern and 30 mls of castor oil; the dog survived the treatment and was killed 8 days later. Dog No. 172, weighing 10 kilos, received 20 mls of oleoresin of male-fern and no castor oil; the dog was very sick and died the sixth night following the treatment. Dog No. 185, weighing 8.5 kilos, received 20 mls of oleoresin of male-fern and 6 grains of calomel; the dog survived the treatment and was killed 9 days later.

The above experiments indicate that oleoresin of male-fern is more dangerous without the administration of a purgative than with the administration of a purgative; that it is more dangerous without purgation than with the use of castor oil as a purgative; that so far as our experiments show anything—and we do not regard these experiments as conclusive, of course—adequate doses of castor oil, promptly administered, are as good in this connection as calomel, and perhaps as good as anything. Without caring to take a radical position in this matter, we are of the opinion that caution in regard to male-fern should emphasize the need of adequate purgation rather than the danger from castor oil. If castor oil were as dangerous as it has been said to be, its use should make a sub-lethal dose lethal by increasing absorption; in actual tests its use makes a dose that is lethal in the absence of purgation non-lethal by aiding in elimination. Whether there is a dose of oleoresin of male-fern of over 20 mls that would be non-lethal to an average-sized dog in connection with the use of some such purgative as a saline and lethal in connection with castor oil, I do not know. However, I am inclined to doubt it. I am inclined to believe that the distributive and purgative action of castor oil

goes far to offset the solubility factor in the case of male-fern. Winslow and many other writers have published prescriptions or given directions for the use of castor oil as a purgative for male-fern, and anthelmintics are marketed which contain both ingredients. As far as solubility is concerned, oil of chenopodium is as soluble in castor oil as male-fern is, perhaps more so, but many experiments with oil of chenopodium indicate that its administration in castor oil gives superior anthelmintic value against some species of worms, at least, and maximum safety to the host animal. In support of this last statement and of the foregoing contentions in regard to the danger in the use of olive oil, the following experiments may be noted:

Dog No. 29, weighing 10 kilos, was given 90 minims of oil of chenopodium in soluble elastic capsules, preceded by 65 mls of olive oil and followed by 35 mls of olive oil. This was in the morning. Examination two and a half hours later showed that the dog was very sick, and it was given an additional 50 mls of olive oil. The dog died that night. Post-mortem examination showed the stomach to be intensely hemorrhagic and the entire intestinal tract inflamed. The dose given was a lethal dose and the 150 mls of olive oil did not protect the dog against it.

Dog No. 29, weighing 10 kilos, was given 90 minims of oil of chenopodium in soluble elastic capsules, preceded by 45 mls of castor oil and followed by 45 mls of castor oil. Examination an hour and a quarter later showed that the dog was sick but sitting up, and it was given an additional 35 mls of castor oil. The dog seemed all right the next day and was killed the fourth day. On post-mortem all the viscera appeared to be normal, except for an inflamed area in the rectum. The dose given was a lethal dose, but the 125 mls of castor oil afforded ample protection against it.

Dog No. 46, weighing 25 kilos, was given 20 mls of oil of chenopodium in soluble elastic capsules, 2 mls of chloroform, and 60 mls of castor oil; 7 days later, this dog was given 75 minims of oil of chenopodium, 5 mls of chloroform, and 60 mls of castor oil. Thirteen days later this dog was given 75 minims of oil of chenopodium in soluble elastic capsules, 5 mls of chloroform, and 60 mls of castor oil. In two weeks, therefore, the dog received 170 minims of oil of chenopodium, or almost 3 drams, in addition to 12 mls of chloroform. The dog was killed on the

twenty-fifth day and the stomach showed a couple of small inflamed areas, the duodenum was slightly congested and the cecum showed two small inflamed areas. I assume that the large dose of castor oil administered to this dog protected it from injurious effects from the chenopodium.

In a series of dogs given oil of chenopodium in therapeutic dose accompanied by olive oil alone or by olive oil and some purgative, almost all of the dogs showed a hemorrhagic condition of the stomach or small intestine on post-mortem, a condition which was rarely met with in post-mortem examination of a large series of dogs given oil of chenopodium in therapeutic dose accompanied by castor oil. It may also be noted that where olive oil alone was given with the chenopodium, the dogs were usually much constipated and often did not pass feces for several days, a condition which must ensure the absorption of practically all the chenopodium given.

5. Anthelmintics, at least some anthelmintics, probably do not need to be allowed "time to act" on the worms before purgatives are administered.

The above proposition is stated tentatively. It is so generally believed and stated that anthelmintics should be allowed time to take effect before any purgation is attempted, that it seems almost unsafe to dispute the proposition. Dock and Bass¹⁶ even explain the failure of remedies to act effectively as possibly due to "the rapid carrying down of the thymol by peristalsis to below the location of the worms," even in the absence of purgation. My own experiences have led me to fear more the absorption of the drug in the stomach before reaching the site of the worms. In over two years' experiment work, involving the treatment and post-mortem examination of over 250 dogs, the results seem to be a little better, if anything, where the anthelmintic and the purgative are administered simultaneously than where the anthelmintic is allowed to precede the purgative by an hour or longer. Such combinations as oil of chenopodium and castor oil, chloroform and castor oil, santonin and calomel, etc., seem to be as effective as the ingredients of the combinations administered separately and at intervals. It is well known, of course, that such anthelmintics as areca nut are themselves purgative and that some ordinary

purgatives are to a slight extent anthelmintic, though not to the extent that they deserve to be called anthelmintics.

Even if it were true that anthelmintics are more effective if purgation is postponed, and it can be shown that the patient is safer where the purgation is given with the anthelmintic, would it not be good practice to repeat a safe treatment oftener than to give a less safe treatment fewer times? Of course, in practice there are other factors involved.

6. Preliminary fasting is important.

This proposition is generally accepted, though an occasional writer seems to regard preliminary fasting as of little importance. Experiments in this laboratory and in the U. S. Bureau of Animal Industry have convinced me that it is bad practice to give anthelmintics to patients with a stomach full of food. The drug is diluted to a point where it exerts a minimum amount of effect on the worms, and sometimes a drug of known potency in adequate therapeutic dose will fail to bring away any worms when given to experiment animals under these conditions. During gastric digestion there is a hyperemic condition of the gastric mucosa and the absorption processes are active, so that a certain amount of the drug is probably absorbed at this point. Such of the anthelmintic as leaves the stomach with the chyme is too diluted, and the worms themselves are too well screened by the mass of partly digested food to permit of effective anthelmintic action. In our experiments the evidence on this point has been convincing. Attempts to treat animals like sheep and swine by the administration of anthelmintics in the food have resulted in failures or indifferent successes, and it appears that such a line of treatment would have to be prolonged and repeated over long periods to attain any reasonable success, with all that such repetition might involve in the way of cumulative toxic results. In a recent paper the writer¹⁷ has pointed out that the desirability for fasting before anthelmintic treatment was not due, as has been often stated and too commonly believed, to the parasites becoming starved and so ingesting the anthelmintic more readily.

7. Gastric stasis might occasionally interfere with the efficacy of anthelmintics.

I have stated elsewhere¹⁸ that "Nothing known to us in the way of vermifuges can be depended on to remove all worms

present, even in repeated doses, in all cases, as there are conditions little understood which at times appear to inhibit the action of even reliable vermifuges." Some of the post-mortem findings on experiment dogs in this laboratory suggest that the failure of anthelmintics might at times be due to gastric stasis. An occasional animal is found with a large, distended, atonic stomach and occasionally dogs are found with a stomach that is apparently normal in appearance, but containing food known to have been fed one or two days previously. It would appear that in such cases anthelmintics might lie in the stomach for long periods or be slowly absorbed there; that where the anthelmintic remained unabsorbed by the atonic mucosa it would sooner or later be diluted by the food and drink subsequently ingested by the animal and so rendered ineffective. It should be admitted, however, that in our cases anthelmintic treatment was usually as effective as could be expected, though the fact that the anthelmintic in these cases was given with a purgative may explain its prompt passage even from an atonic stomach and its subsequent efficacy. I believe gastric stasis might be reasonably invoked as the explanation for occasional failure in anthelmintic medication, especially where purgation follows treatment at a long interval and where such depressant or constipating drugs as oil of chenopodium are used.

8. The passage of worms following anthelmintic treatment is a better indication for repeating or continuing treatment than for stopping treatment.

As has been noted previously in this paper, the action of oil of chenopodium against ascarids is the only case in which we have commonly found approximately 100 per cent efficacy against worms in single, therapeutic dose of an anthelmintic, and this drug will occasionally fall below this point in efficacy. In common practice too many medical men regard the passage of worms as evidence that the treatment has done its work, whereas the real test of an anthelmintic is to be made on the basis of the worms not removed. A patient may pass a large number of worms, and have a much larger number left. When a well-selected treatment finally fails to bring away worms it establishes a fair presumption to the effect that all worms of the sort involved have been removed. This, of course, should be checked up by a consideration of the persistence or disappearance of symptoms, and, even more,

by microscopic examination of the feces for parasite eggs. The latter should extend over a period of perhaps two weeks after treatment and should cover several days in that period if accurate information is desired, as it seems well established that anthelmintic treatments may inhibit worm egg production for some time, and it requires several negative examinations of feces to establish a fair presumption in favor of freedom from infestation where infestation is suspected or known to have existed.

9. Severe helminthiasis calls for caution in administering anthelmintics.

In other words, severe helminthiasis, with a feeble, emaciated patient suffering from enteritis as a result of numerous worms and with the bodily resistance already much lower as a result of worm toxins, is itself a contraindication for anthelmintic treatment both as regards the production of local irritation in the digestive tract and the production of toxic systemic effects. More than once I have had weak experiment animals heavily infested with worms die soon after the administration of an anthelmintic. It is quite likely that these animals would have died very soon as the result of worm infestation, but it was evident that the ordinary plan of attack, suitable for reasonably strong animals with moderate infestations, was not suitable for these animals. It has been recognized in dealing with hookworm disease in man that bad cases, with pronounced anemia and well-developed clinical symptoms of the disease, must be treated with caution. The value of using repeated small doses of anthelmintic in such cases has been emphasized by Stiles and Leonard¹⁹, and various writers have noted the need for preliminary building up of the bodily resistance by nutritious food. In the field of human medicine there is no disposition to overemphasize this factor, but in the field of veterinary medicine it is not an uncommon thing to find writers who regard food as of more importance in cases of parasitic infestation than anthelmintics. This phase of therapeutic nihilism is explained by its advocates on the grounds that parasites do not seem to thrive in thrifty animals; the more obvious statement of this condition would be that thrifty animals are thrifty because, for one thing, of their freedom or comparative freedom from parasites. Therapeutic nihilism in the field of anthelmintics has little to sustain it. While the drugs used in this

capacity are in need of much more study, they nevertheless include a number of demonstrably potent drugs serving a purpose that cannot be served by any means other than medicinal, except by way of prophylaxis.

10. While the majority of worms passed after anthelmintic treatment come away in the first 24 hours after treatment, there is a fairly large per cent which will commonly come away from one to six or seven days later.

This delay is perhaps due to a poisoning of the worms which weakens or sickens them to the point where they are brought away some time after the anthelmintic treatment, or in other cases it appears to be due to intestinal stasis, which may not be overcome by the purgation employed. In our series of experiments on anthelmintics in this laboratory, using dogs as experiment animals, we found the following to be true where single treatments, not involving more than a few hours of one day, were given:

a. *Ascarids*: Approximately 82.7 per cent were passed during the day after treatment, *i.e.*, within 24 hours after treatment; 7.7 per cent were passed the second day after treatment, *i.e.*, within the second 24 hours; 4.3 per cent were passed the third day after treatment; 3.1 per cent were passed the fourth day; 1.5 per cent were passed the fifth day; 0 per cent were passed the sixth day; 0.5 per cent were passed the seventh day.

b. *Hookworms*: Approximately 74.1 per cent were passed the first day after treatment; 15.7 per cent were passed the second day; 7.4 per cent were passed the third day; 2.8 per cent were passed the fourth day; 0 per cent were passed the fifth, sixth and seventh days.

c. *Whipworms*: 57.6 per cent were passed the first day; 15.2 per cent were passed the second day; 18.2 per cent were passed the third day; 10 per cent were passed the fourth day; 0 per cent were passed the fifth, sixth and seventh days.

d. *Dipylidium spp.*: 91 per cent were passed the first day; 7.4 per cent were passed the second day; 0 per cent were passed the third day; 1.6 per cent were passed the fourth day; 0 per cent were passed the fifth, sixth and seventh days.

e. *Tenia spp.*: 100 per cent were passed the first day; 0 per cent were passed the second to the seventh days inclusive.

The number of experiments involving *Tenia* is really too

small to warrant any general conclusions, but, accepting them on a par with the other findings, we may summarize the foregoing as follows:

For all worms concerned, after one anthelmintic treatment, from 57.6 per cent to 100 per cent of the worms came away the first day; in other words, a majority, over 50 per cent, of those that came away, came away within 24 hours after treatment. Aside from *Tania*, where the per cent for the second day drops to zero, there came away during the second day, from 7.4 per cent to 15.7 per cent of the worms that were removed. During the third day, or the third 24-hour period, there came away from 0 to 18.2 per cent of the worms removed. During the fourth day, there came away from 0 to 10 per cent of the worms. During the fifth day, there came away from 0 to 1.5 per cent of the worms. There were no worms passed on the sixth day. On the seventh day, there came away from 0 to 0.5 per cent of the worms passed. It must be understood that these figures are merely a statement of the findings of our experiments, not at all intended as a statement of a rule, but they suggest that the physician who takes into account the feces for only 24 hours after treatment may overlook things of importance.

The number of worms passed diminished each day with *Tania* and hookworms; it diminished to zero on the sixth day with ascarids, but rose on the seventh day slightly; it diminished to zero on the third day with *Dipylidium*, but rose slightly on the fourth; it diminished on the second day with whipworms, but rose on the third, diminishing from there on to zero on the fifth day. An examination of the figures for these worms seems to warrant the belief that with sufficient figures, all these worms would show a smaller number passed each day with the exception of the whipworm; there appears to be some ground for thinking that there is little difference in the chances of whipworms appearing the second or third day, and it may be that the number would be steadily greater on the third day than on the second, as a rule. The greater per cent of the total number of worms passed which come away the first day is in this order: *Tania*, *Dipylidium*, ascarids, hookworms, whipworms.

REFERENCES.

1. Lutz, Adolph. *Centralb. f. Bakt. u. Parasit.*, 1888, 20, 617-620.
2. Hall, M. C., and W. D. Foster. Oil of Chenopodium and Chloroform as Anthelmintics. *Journal A. M. A.*, June 30, 1917, pp. 1961-1963.
3. Radtke, A. *Rec. d. med. vet.*, 1915, 15, pp. 490-513.
4. Stiles, C. W. *Public Health Repts.*, 1917, 33, pp. 1299-1301.
5. Lane, Clayton. *Indian Med. Gaz.*, 1915, 7.
6. Hall and Foster. *Loc. cit.*
7. Hall, M. C. Animal Parasites, in Musser and Kelley's Practical Treatment, v. 4, pp. 389-419.
8. Berard and Vignard. *Med. Fortnightly and Lab. News*, 1916, 11, 339-345.
9. Bozzolo, Camillo. Notes on the Treatment of Ankylostoma Anemia (Uncinariasis, Hookworm Disease) with Thymol. *Journal A. M. A.*, June 8, 1912, p. 1744.
10. Schultz, W. H., and A. Seidell. Orig. Comm., 8th Intern. Cong. Appl. Chem., 1912, 19, p. 281.
11. Seidell, A. Hyg. Lab. Bull. 101, 1915, III, pp. 43-51.
12. Salant, Wm., and A. E. Livingston. *Proc. Soc. Exp. Biol.*, V. 12, 6, p. 130.
13. Salant, Wm., and E. K. Nelson. *Am. J. Physiol.*, 1916, 4, pp. 440-463.
14. Agnis, E. J. *Proctol. and Gastroenterologist*, 1917, 2, pp. 86-90.
15. Winslow, Kenelm. *Veterinary Materia Medica and Therapeutics*, N. Y., 1913, 799-195.
16. Dock, Geo., and Charles C. Bass. *Hookworm Disease*, St. Louis, 1910, 250 pp.
17. Hall, M. C. The Longevity of Adult Ascarids Outside the Body of the Host; Its Bearing on Anthelmintic Treatment. *Journal A. M. A.*, March 10, 1907, p. 772.
18. Hall, M. C. In Musser and Kelley, *loc. cit.*
19. Stiles, Ch. W., and G. F. Leonard. *Pub. Health Repts.*, 1911, 42, p. 1925; 1913, 3, p. 119.



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**A NOTE REGARDING MYIASIS, ESPECIALLY THAT
DUE TO SYRPHID LARVAE.**

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Bruce¹ (1917) has recently published a paper reporting from Vancouver Island a very interesting case of vaginal myiasis in the cow due to the "rat-tailed larva" of the drone fly, *Eristalis*. He does not say how many larvæ were found, but notes that the vagina was diseased and that a discharge was present. In his paper, Bruce says: "I am unable to find any reference to invasion of the vaginal cavity by the larvæ of *E. tenax* in either man or animals." As a matter of fact, Bruce's case is the second recorded case of this sort, the first having been published by Hall and Muir² (1913). Their record is as follows:

In the Bureau of Animal Industry collection of parasites . . . there are also eight *Eristalis* larvae sent in from Laurel, Md., in 1909, with the statement that they were passed in a jelly-like substance from the vaginae of cattle. In correspondence relative to these specimens, Dr. B. H. Ransom suggests to the sender:

Probably a diseased condition of the organ in which you found these larvae created an odor which attracted the flies to this particular place, with the result that they have deposited their young in the unusual location.

The above record has been cited by Metcalf³ (1916), but it is not at all surprising that the record escaped the attention of Dr. Bruce. The fact that we now have two records of the presence of *Eristalis* larvæ in the diseased vaginae of cattle instead of one record, however, does much to establish the validity of both records and the likelihood that similar occurrences do happen from time to time.

¹Bruce, E. A.: A New Parasite for Cattle. The Larvae of *Eristalis tenax* L. (drone fly), Jour. Am. Vet. Med. Assn., 1917, 1, 66-68, Fig. 1.

²Hall, M. C., and Muir, J. T.: A Critical Study of a Case of Myiasis Due to *Eristalis*, The Archives Int. Med., 1913, 11, 193.

³Metcalf, C. L.: Syrphidae of Maine, Bull. 255, Maine Agric. Exper. Sta., 1916.

Hall and Muir¹ listed a summary of seven published cases of myiasis in man due to larvae of the *Syrphidae*. To this they add their new case and four cases from the files of the United States Bureau of Entomology, furnished through the courtesy of Dr. L. O. Howard and Mr. R. S. Clifton, and one from the files of the United States Bureau of Animal Industry, furnished through the courtesy of Dr. A. D. Melvin and Dr. B. H. Ransom, to whose courtesy they were also indebted for the record of the case of vaginal myiasis. Twelve of these were cases of gastrointestinal myiasis and one (Leidy's) a case of nasal myiasis. To the summary of human cases by Hall and Muir, I wish to add an unpublished case in man, for which I am indebted to the kindness of Dr. Allen J. Smith of the University of Pennsylvania and Dr. E. J. Bardwell of Tunkhannock, Pa., and to note a subsequently published record by Ichess² (1914), and three records of Austen (1912, not available), cited by Graham-Smith³ (1914).

Under date of April 24, 1913, Dr. Smith wrote me as follows:

I have recorded here in our laboratory reports a case of *Exastasis* in the human stool, definitely regarded as *Exastasis*, and tentatively, for the same reason you assign in your case, *E. tenax* (common occurrence of the fly). The specimens (two) were sent Sept. 9, 1908, by Dr. F. J. Bardwell of Tunkhannock, Pa., through the State Health Department (the bacteriological work of which was then done by this laboratory) as from the stool of B. L., white, male, native, aged 18. No statement as to the condition of the subject and no account of the circumstances of recovery of the specimens were given, although doubtless Dr. Bardwell can furnish them. I note the specimens as 13 and 14 mm. in length, respectively, 4 and 5 mm. thick in anterior part, the "tail" as equaling the body length in the first, somewhat retracted in the second; tail curved anteriorly. Dark brown, firm, apparently brittle, seven pairs of hook-bearing prolegs (numerous hooks arranged in curved rows). Dorsally at head end two pairs of hooks, the posterior the longer and directed forward, the anterior the smaller and directed backward. On each side submedian row of indomite tubercles, between which are a number of fine transverse ridges of the cuticle. Indefinitely seven-segmented. . . . It has never been published.

In response to a recent request for information in regard to this case, Dr. Bardwell wrote me, under date of Oct. 12, 1917, as follows:

I remember the case you refer to very well. . . . About a year

¹ Trans. Entom. Soc. Amer., 1909, 1: 1-10, tabular record, Aesch. d. parentalis, 1914, 3.

after that, he had the same thing again. There is no doubt about it being a genuine case. Creolin, one dram to quart of hot water, was the treatment. The symptoms are intense irritation of the rectum.

Iches¹ (1911) states that when traveling in the Argentine Republic he found in the entomologic collection which is under Dr. Lahille's supervision, two rat-tailed larvae collected at Necochea in 1904, and was furnished the following history by Dr. Lahille:

A physician at Necochea was called in to attend a 7-year-old girl who complained of a pain in the rectum. Nothing was detected on simple examination and the doctor ordered washing, which brought about no change. He then gave a purgative, which caused the expulsion of the larvae, which were sent to Lahille. The two larvae measured as follows: Body 12.5 mm. long by 2 mm. wide; tail 18 mm.; body 11 mm. long by 2 mm. wide; tail 13.5 mm. The color was dirty gray.

Austen's cases of human myiasis due to syrphid larvae are noted by Graham-Smith² as follows: "In England, Austen (1912) has met with . . . two cases due to the larvae of a *Syrphus* or hover-fly, one case due to the 'rat-tailed maggot' of *Eristalis tenax*. . . ."

It would appear, then, that there are at least seventeen records claiming the presence of syrphid larvae in the digestive tract of man; one record claiming their presence in the nostrils of man; and two records claiming their presence in the diseased vaginae of cattle.

In passing, it might be noted that Blundeville in his book, "The Order of Curing Horse Diseases," published in 1609, says that a "bottee" has a "great head and small long tail like a needle." This is a very poor description of a bot and a fairly accurate description of a rat-tailed larva. It suggests that Blundeville had seen rat-tailed larvae in horse manure, a favorite breeding place, and regarded them as bots which had been passed by the horse, and this may be a rather common error. It is still a common error to regard free-living forms occurring in feces as parasites which have passed from the digestive tract. Herms³ (1915) says that the frequency with which the larvae occur in liquid excrement must lead to caution in accepting reports that they have been

¹Graham-Smith, G. S.: Flies in Relation to Disease. Nonbloodsucking Flies. Cambridge, 1914.

²Herms, W. B.: Medical and Veterinary Entomology. New York, 1915.

evacuated, and adds: "The writer has on several occasions received specimens of 'rat-tailed' larvae which were said to have been evacuated by the 'double handful' and that the patient had 'steadily improved' thereafter."

As regards the species of syrphid involved in these cases, we may quote Metcalf's opinion:

I wish to emphasize the practical impossibility, at our present state of published knowledge, of referring larvae found in such circumstances to a definite species, or even to the genus, unless specimens are reared to the adult. It seems to be the custom to refer any rat-tailed larva to *Eristalis tenax*, or at least to the genus *Eristalis*. Such records, unless based on adults reared from the larvae, must, it seems to me, be discarded as of no specific importance. I . . . have examined a dozen species of rat-tailed larvae belonging to several genera, the separation of which is exceedingly puzzling and difficult and any one of which might easily be mistaken for the larvae of *Eristalis tenax*.

Hall and Muir stated in regard to these rat tailed larvae: "Only an occasional pupa, and never a larva, survives the winter." Regarding this point, Mr. E. R. Warren of Colorado Springs, Colo., a well known ornithologist and mammalogist, wrote me, under date of March 18, 1913, as follows:

The latter part of February, 1902, I took from an outdoor tank at a fish hatchery near Crested Butte, Colo., a bunch of live rat tailed maggots, which were identified by Dr. L. O. Howard as *Eristalis tenax*. He made no comment as to the season when they were found, and perhaps did not understand that they were taken in winter. . . .

The tank was a wooden affair, perhaps 4 by 8 by 3 feet deep, in open air, not used for any special purpose, but the water from the hatchery flowed through it. This water is taken from a spring and piped right into the building and flows through the hatching troughs, and hence out into the tank I have mentioned. . . . As the winters are cold there, 9,000 feet, the temperature of the water in the tank was probably not much above 32 F., but as it comes from the spring into the hatchery it is 45 F. the year around.

One sort of myiasis about which little of a specific sort seems to be published is rectal myiasis, due to blow fly larvae, especially in sick and in moribund animals, and more especially where there is blood in the feces. I have seen a few cases of this sort and had one case in the dog recently. In this case the dog was being used to determine the lethal dose of an anthelmintic, and a resultant hemorrhagic gastritis was the source of the blood in the

feces. The day before this dog died, maggots were found to be crawling in and out of the anus. To alleviate this condition, the dog was given a rectal injection of 1 c.c. of chloroform in 9 c.c. of castor oil. On postmortem examination the following day, the rectum, colon and anal region were found free from larvæ, but four larvæ, dead when collected, were found in the small intestine. It is possible that these four larvæ had been driven back up the large intestine by the rectal injection and had entered the small intestine either before or after the death of the dog. The dog had eaten nothing for several days, except a small amount of milk, and it appears more likely in this case that the larvae in the small intestine entered the body by way of the anus than by way of the mouth. It suggests that occasional cases of gastric and enteric myiasis might be explained in this way in cases in which the evidence seemed opposed to the idea of infection by mouth. Graham-Smith⁵ (1914) has noted this as a possible mode of infection in man. He says that "Babies left exposed in an uncleanly condition may become infested. The larvae on hatching make their way into the rectum, and perhaps penetrate into the intestine."

**Studies from the Research Laboratory,
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Reprint No. 152, 1918.**

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**BACTERIOLOGIC FINDINGS IN OZENA—SECOND
REPORT.***

BY HERBERT C. WARD AND DONALD C. BEAVER, DETROIT, MICH.

The etiology of chronic catarrhal infection demands more serious attention by the bacteriologist than it has yet been given. Available records repeatedly indicate that invasion of the upper respiratory tract may take place very early in life, children of three and four years becoming subject to pronounced forms of rhinitis from which they never recover. Our clinics register daily the ozenic and atrophic, the tuberculous or syphilitic adult of forty years. In fact there is no lack of fresh material or willing patients for the needs of serious investigation. Both are abundant from various sources and climates and of all ages.

In tabulating the cases coming to this laboratory for observation we have found it convenient to make the following distinctions. The diagnosis of atrophic rhinitis is indicative of atrophy, chronicity of discharge, crustal formation, and impairment of free nasal respiration. The term ozena refers to the obvious symptom of malodor. Inasmuch as ozena is closely associated with atrophic rhinitis and frequently presents all the symptoms of the same, it is here considered as a frequent symptom of atrophic rhinitis, and not ozena, a clinical entity. By definition, therefore, causative factors of the atrophic and also of the ozenic stage may not be identical. Investigation of this constitutes the objective of our study on some seventy cases. One hundred and fifty bacteriologic analyses were conducted as completely as the time and facilities were convertible for this work. The findings agree with those of the first paper† and have yielded very suggestive records.

The most comprehensive report published in this country is that of Horn of San Francisco. He suggests that ozena is not a

*From the Research Laboratory of Parke, Davis & Company, Detroit, Mich.

†Bacteriologic Findings in Ozena, First Report, *Jour. Infect. Dis.*, Aug., 1916, xiv, 153.

clinical entity, meaning that more than one biologic agent may bring this condition about. After reviewing previous work and supplementing the same with a series of experimental vaccines,

TABLE I.

<i>B. mucosus capsulatus</i> (Abel's bacillus)	appeared in 42 cases, or 84%
<i>B. diphtheroids</i>	" " 37 " " 74%
<i>M. staphylococci</i>	" " 37 " " 74%
<i>B. Perez Type II</i> (atypical)	" " 34 " " 68%
<i>M. streptococci</i>	" " 31 " " 62%
<i>B. proteus</i>	" " 14 " " 28%
<i>B. Perez Type I</i> (typical)	" " 11 " " 22%
<i>B. influenzae</i>	" " 9 " " 18%
<i>M. pneumococci</i>	" " 8 " " 16%
<i>M. catarrhalis</i>	" " 6 " " 12%
<i>B. coli</i> and <i>pyocyaneus</i>	" (each) 3 " " 6%

he proceeds to show that bacteriologically all his cases fall into two groups, one designated as the "Friedlander" and the second as the "Perez" group. Literature of the subject reveals similar conclusions from the earliest of the investigations and a previous report from this laboratory records identical findings.

Bacteriologic studies have aimed to include the most important groups of organisms so far as routine analyses and methods would permit. Table I includes the findings obtained from fifty cases diagnosed as ozema (atrophic rhinitis fetid). The groups are arranged in the order of their predominance in the fifty cases.

These cases are representative of conditions found in this country, having been taken from a wide radius as follows: Michigan, 25 cases; Louisiana, 5; Illinois, 2; Minnesota, 3; Ohio, 7; California, 4; and Kentucky, 4. Inasmuch as the material had to be shipped a long distance before being subjected to bacteriologic examination, the thoroughness of the analyses was limited to but one trial in the majority of the cases.

Reviewing Table I, it is evident that the preponderance of *B. mucosus capsulatus* is most significant. An organism similar to this and known as the Morax-Axenfeld bacillus appeared only a few times and is not, therefore, considered as being important. It is easily confused with the *B. mucosus capsulatus* microscopically, but never culturally.

Diphtheroid bacilli have taken second place in the table. In

two instances toxin-producing bacilli were abundantly present and the cases were classed as diphtheria carriers by the local board of health. The identification of such organisms from patients suffering from chronic catarrh is most suggestive for practical investigation bearing on the problem of diphtheria carriers. No work of this character has as yet been called to our attention.

The much discussed Perez group of bacilli has appeared in a higher percentage of cases than in the first census. The strains isolated do not conform, on careful study, to the reported type. Two groups are distinguished. Type I represents a class conforming to the standard strain. Type II represents atypical Perez strains, differing from the stock in their agglutination, fermentation, and motility tests. Neglecting the question of strain identity for the moment, the significant fact appears that some representative of the Perez group is found in forty-five cases out of the fifty. Of additional value is the discovery that those cases contain no Perez types, but in their place, bacilli set down as *B. proteus vulgaris*. The Perez and the proteus groups are, therefore, responsible for the malodor of ozena cases.

The remaining groups of organisms appear at present to be of no significance.

Supplementary to the fifty ozena cases just reviewed, the following group, diagnosed as "atrophic rhinitis," and differing from the former only by the absence of odor, is tabulated. Material for bacteriologic study was received from: Michigan, 14 cases; Kansas, 1; Minnesota, 2; Illinois, 1; Ohio, 1; Indiana, 1; and Wisconsin, 1—making a total of 21.

TABLE II.

<i>B. mucosus capsulatus</i>	appeared in 17 cases, or 81%
<i>M. staphylococcus</i>	" " 16 " " 76%
<i>M. streptococcus</i>	" " 14 " " 70%
<i>B. diphtheroids</i>	" " 12 " " 60%
<i>B. influenzae</i>	" " 3 " " 15%
<i>M. catarrhalis</i>	" " 3 " " 15%
<i>B. spore bearers</i>	" " 2 " " 10%
<i>B. pyocyaneus</i>	" " 2 " " 10%
<i>B. Morax-Axenfeld</i>	" " 1 " " 5%
<i>B. Perez</i>	" " 1 " " 5%
<i>B. proteus</i>	" " 1 " " 5%

Two facts are strikingly evident: first, that the capsulatus group is the most abundant; and, second, that the Perez and the proteus representatives are the least abundant. In this connection it is to be noted that the patients when examined at the clinics showed no odor. The nasal discharges, when incubated, developed no odor (except in two cases) and yielded no cultures giving odors. The results suggest that the existence of a Perez-proteus infection can be recognized in the majority of cases by clinical examination alone, and in such instances bacteriologic examination is necessary.

A summary of our study shows that the group of organisms known as the *B. mucosus capsulatus* (Friedlander's bacillus, Abel's bacillus) is preponderant and may be pathognomonic in cases of chronic rhinitis. This group is also independent of the action of putrefactive bacteria. In cases in which the symptom of malodor exists, a different class of bacilli known as the Perez group is most abundant, and all cases show either one or both of the Perez and proteus groups present as causative factors of this condition.

Cases of chronic catarrh may harbor pathogenic organisms of other species such as the diphtheria bacilli and contribute to the distribution of similar infections.

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STUDIES ON DIPHTHERIA TOXIN.*

BY LEWIS DAVIS, S.M., DETROIT, MICH.

I.—HYDROGEN-ION CONCENTRATION AND TOXICOGENICITY DETERMINATIONS WITH BACT. DIPHTHERIÆ.

Historical Resume.—Some of the earliest workers with Bact. diphtheriæ have noted that the reaction of the culture medium has a decided influence on growth and toxin production with this organism. Roux and Yersin,¹ who appear to have been the first to extensively investigate this question, recommend the use of slightly alkaline bouillon, since they found that an acid reaction did not permit of strong toxin formation. Spronck² attributed this acid production to the presence of a variable quantity of glycogen and glucose, fermentable by Bact. diphtheriæ, in the meat used for the bouillon. To overcome this factor, he proposed the employment of decomposed beef or veal in which the putrefaction had reduced the content of these sugars to a minimum.

Park and Williams³ found that with the beef used by them, the inhibitory action of the muscle sugar was neutralized by adding sufficient alkali to the bouillon (about 1 c.c. of normal soda solution per liter). They state "that an excessive amount of either acid or alkali prevented the development of toxin" and that the "type of growth of the bacilli and the rapidity and extent of the production of toxin depended more on the reaction of the bouillon than upon any other single factor."

Essentially the same results have been found by Madsen.⁴ This investigator concludes that in the same bouillon, diphtheria cultures can develop in one of two directions, acid or alkaline, depending upon the initial degree of alkalinity. Confirming the results of Spronck, and Park and Williams, acid cultures were found by him to possess no toxicity, while, in general, the alkaline cultures are toxic, although no definite relationship exists between the degree of alkalinity and the amount of toxin produced.

*From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.

In order to eliminate any action of the muscle sugar, Theobald Smith⁵ proposed a peptone bouillon in which the beef infusion, previously reduced in acidity, was submitted to a preliminary fermentation overnight with *B. coli*, then sterilized and filtered. This would break up all carbohydrates, and give at once an alkaline growth with a constant, strong toxin. Addition of dextrose to such bouillon in quantities not exceeding 0.2 per cent, was found not only to be uninjurious, but actually led to a maximum accumulation of toxin, presumably by utilizing the available peptone to the best advantage. This method has not met with general favor, although Hitchens⁶ reports good results from its use with veal bouillon. Lubenau⁷ confirms the findings of Smith that no acid is formed in carbohydrate-free, nutrient bouillon, and also states that, as a rule, diphtheria bacilli produce alkali in such bouillon independent of the initial reaction. On the other hand, he finds that diphtheria-like organisms produce no appreciable amount of alkali, but leave a carbohydrate-free bouillon unchanged. In ordinary bouillon, which would contain carbohydrates, both diphtheria and diphtheria-like organisms produce acid, regardless of the initial reaction of the bouillon.

Jacobsen,⁸ in a critical review of the work of Mad-sen and of Lubenau, agrees that in sugar-free bouillon (Smith method) diphtheria cultures produce alkali at once. He also finds that, not only has dextrose an influence on the acid formation, but with a constant amount of glucose present in a bouillon, the amount of acid produced increases proportionately to the peptone content. Any culture of *Bact. diphtheriæ* in bouillon can, according to him, theoretically be considered as passing through the following stages: primary acid formation, reversal, alkali formation, secondary acid formation. This last stage which takes place only after a prolonged period of time (two to four months) has its origin in the neutralization products of the above mentioned reversal stage and is different from the primary acid formation which is attributed to the muscle sugar present.

A consideration of the work mentioned above, in the light of the modern conception of reaction based on hydrogen-ion concentration, brings up at once the limitations of results obtained by the usual titrametric methods. In fact, it is now generally conceded that there is a decided difference between the acid and alkaline values obtained by titration using litmus and phenolphtha-

lein. Clark,⁹ in an able discussion of the fallacy of titrating hot media, states, "It is needless to add that since titrations in the last analysis are based upon the attainment of a certain hydrogen-ion concentration as shown by the tint of an indicator, the titration of a medium at 90° to 100° C. furnishes data of no exact significance at ordinary incubation temperatures."

In connection with the development of a satisfactory bacteriologic peptone, I¹⁰ have employed the production of a potent diphtheria toxin with a trial sample as one of the biologic tests. This required a study of the factors influencing growth and toxin formation in bouillon of constant composition and, as would be expected from the preceding resume, the hydrogen-ion concentration of the medium has been found to be of prime importance. The present investigation was undertaken to determine more accurately by means of the hydrogen electrode what reaction changes take place in the medium during the propagation of diphtheria toxin on a practical scale, and to note what relationship, if any, exists between toxicogenicity in a medium and hydrogen-ion concentration.

LABORATORY DATA.

Methods of Experimentation.—Extensive experience has shown that plain bouillon gives uniformly satisfactory results in the production of toxin with Bact. diphtheriæ. Sugar media and carbohydrate-free broth (Smith method) have not proved of any advantage. In the study at hand, unless otherwise noted, the medium employed was plain bouillon, made up as given in the following:

To every liter of beef infusion prepared in the usual manner, 20 grams of peptone (Bacteriologic, Parke, Davis & Co.) and 5 grams of sodium chloride were added. Supplies of sterile beef infusion and of the other two ingredients from individual lots were retained in quantities sufficient for all of the experimentation so as to eliminate any variations of the bouillon components. The peptone and salt were first dissolved in the cold, then heated in a steamer for fifteen minutes to insure thorough solution.

The medium was now ready for preliminary adjustment to the desired hydrogen-ion concentrations which was done essentially as proposed by Clark and Lubs,¹¹ using the buffer solutions, standardized by the hydrogen electrode, and the indicators recom-

mended by them. As will be later shown, the limiting concentration values for cultivation of the diphtheria bacillus are approximately $C_H = 1 \times 10^{-8}$ ($P_H^+ = 6.0$) and $C_H = 1 \times 10^{-9}$ ($P_H^+ = 9.0$), respectively, which reduces the standard buffer solutions necessary to the "acid potassium phosphate-sodium hydroxide" mixtures and the "boric acid-potassium chloride-sodium hydroxide" mixtures. "Brom cresol purple" and "brom thymol blue" were used as indicators for the media on the acid side; "brom thymol blue" for those concentrations around true neutrality, while in the alkaline zone, "phenol red," "cresol red," and "thymol blue" were employed. The various media were then neutralized essentially as described below for regular diphtheria toxin bouillon, the final ion concentration being determined electrometrically.

For the routine production of diphtheria toxin, in bouillon, hydrogen-ion concentrations around 1.0×10^{-8} ($P_H^+ = 8.0$) have been employed with excellent results. Such reaction values are rapidly obtained by taking, for the preliminary adjustment, 10 c.c. of the medium, after the first heating, diluting with about 40 c.c. of distilled water, and titrating against dilute ($N/10$) sodium hydroxide in the cold, using phenolphthalein as the indicator. A little experience will soon determine what shade of pink should be taken as the end point. The requisite amount of strong sodium hydroxide to entirely neutralize ($10N$ is recommended), in accordance with the preceding titration, is now added slowly and with thorough mixing to the bouillon. The medium is next heated in flowing steam for 30 minutes, or boiled for five minutes, which brings down a heavy precipitate that rapidly settles.

The reaction of the clear, supernatant liquid, or a dilution of it with "conductivity" water, is now, for very accurate work, checked by the hydrogen electrode. Ordinarily, however, a colorimetric "check" by means of the simple comparator of Hurwitz, Meyer and Ostenberg¹² and using standardized mixtures (of $P_H^+ = 7.8, 8.0$, and 8.2) with "phenol red" will be found sufficiently accurate and more rapid. In my experience, the hydrogen-ion concentration of media prepared as above, falls within a range covered by $C_H = 1.5 \times 10^{-8}$ to $C_H = 7.0 \times 10^{-9}$ and may be used for diphtheria toxin production without any further adjustment. The bouillon is now filtered hot, distributed as desired, and sterilized. All of the media used in the present investi-

gation have been sterilized once for 20 minutes at 115° C. (15 pounds steam pressure).

Comparative toxicity determinations have shown that more satisfactory results are obtained by using larger flasks for cultivation of *Bact. diphtheriæ* than the same proportionate amount of bouillon distributed in smaller containers. Accordingly, 3,000 c.c. of the test medium in six liter flasks were employed and inoculations made in each case, with 24 hour "starters" containing 30 c.c. of culture grown in a 250 c.c. flask to accustom the organism to the medium. After culturing for the desired length of time at 37° C., the purity of the growth was checked, the samples preserved with 0.4 per cent cresols and filtered through unglazed porcelain.

In the preliminary experimentation on the ion concentration changes produced during growth of *Bact. diphtheriæ*, use was made of the large flasks fitted with special syphons, so that samples could be removed aseptically from time to time as desired. Control flasks, without syphons, showed that this procedure so interfered with the growth through disturbance of pellicle formation as to entirely vitiate the value of any results obtained. A sufficient number of flasks has accordingly been employed to enable triplicate determinations of any desired value. In practically every case it has been found possible to obtain duplicate hydrogen-ion concentration and toxicity results which checked within practical limits. The toxicity valuations, made in association with my colleague, T. Ohno, were, unless otherwise noted, determined for L^{+} dose on samples in duplicate.

The electrometric determinations of hydrogen-ion concentration were made with the chain:

Calomel electrode (N KCl) — saturated KCl — test medium — Pt Electrode — (H_2) at 23° C. The complete "set up" employed is shown in Fig. 1. C is a "normal" calomel electrode which dipped into the small vessel D containing saturated KCl to reduce to a minimum, the contact potentials between the N KCl and the liquid under examination. Connection between D and the test fluid in electrode vessel A was maintained by a wick saturated with KCl , passing through a small glass syphon tube H . Hydrogen electrodes (B) of the type described by Bovie,¹² were employed, supplied with purified hydrogen, generated from the electrolysis of $NaOH$ with nickel electrodes. The temperature of the liquid

in A was determined by a thermometer accurate to $\pm 0.1^\circ \text{C.}$, and a similar thermometer was mounted near the calomel electrode C .

The hydrogen-ion concentration was read directly by means of the "ionometer" (E) described by Bartell.¹⁴ The known potential derived from a Weston Standard Cell (S) (calibrated by the Bureau of Standards) was first brought into the circuit through the double-pole, double-throw switch F , after which sufficient resistance (4821 ohms) was thrown in to compensate for the calomel electrode employed. With the pointer arms of the logarithmic and concentration coils set at the proper values (in this case 3.0 and 10^{-10} respectively) the external resistances, to compensate for the storage battery, were now adjusted until no deflection was noted in the galvanometer (G), which indicated that the instrument was in balance for direct reading. The resistance values thus obtained were found to remain practically constant for several hours with a good accumulator.

By reversing F the unknown cell consisting of hydrogen electrode and calomel was next connected in; the other resistances remaining the same, and readings could now be obtained directly in terms of hydrogen-ion concentration by manipulating the concentration and logarithmic coils until the galvanometer showed a balance. The ionometer and galvanometer were mounted so as to insure proper insulation from stray currents and freed as much as possible from tremors. During all of the measurements, the room temperature was such that no difficulty was experienced in keeping the thermometer practically constant at 23°C.

Experimental Protocols. Before making a study of the toxicogenic and hydrogen-ion concentration changes produced by the growth of *Bact. diphtheriae* in bouillon, the question arose as to what is the optimal zone of ion concentration for such metabolic activities. This made it necessary to determine within what limits of acidity and alkalinity a known, toxicogenic culture of *Bact. diphtheriae* can produce toxin of prescribed strength. For this purpose there were made up, as already described, lots of bouillon varying in reaction and sufficient in amount to permit of triplicate determination with large flasks at each ion concentration. The flasks were now inoculated with "starters" of a *Bact. diphtheriae* culture (No. 036) originally obtained from W. H. Park and capable of elaborating a toxin in standard bouillon of which one

L+ dose is less than 0.25 c.c. Cultivation of each set was carried on at 37° C. for two weeks and at the end of this period samples were removed from each flask for determination of the final

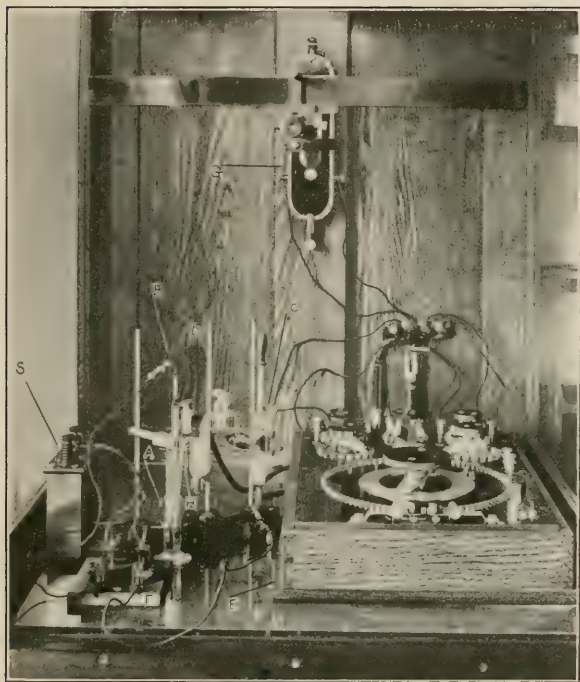


Fig. 1.

hydrogen-ion concentration. The remainder of the bouillon was then preserved and set aside for toxin estimation. The results obtained are summarized in Table I.

TABLE I

EFFECT OF VARYING INITIAL CONCENTRATIONS ON GROWTH AND TOXICOGENICITY OF BACT. DIPHTHERIE

INITIAL C ₀	CHARACTER OF GROWTH	FINAL C ₀ (2 WEEKS)	TOXICITY 1 x DOSE
6.4×10^7	Very scant	4.2×10^8	More than 2.0 c.c.
1.0×10^7	Moderate	7.0×10^8	Between 1.5 and 2 c.c.
7.5×10^6	Heavy	2.1×10^8	1.0 c.c.
1.0×10^7	"	2.0×10^8	0.7 c.c.
7.0×10^8	Very heavy	1.8×10^8	Less than 0.25 c.c.
3.6×10^8	" "	1.6×10^8	" " 0.25 "
2.0×10^8	" "	9.7×10^7	" " 0.25 "
1.0×10^8	" "	7.6×10^7	" " 0.25 "
8.5×10^7	" "	7.2×10^7	" " 0.25 "
7.0×10^7	" "	6.9×10^7	" " 0.25 "
5.0×10^7	" "	4.2×10^7	" " 0.25 "
2.0×10^7	" "	3.0×10^7	" " 0.25 "
1.1×10^7	Moderate	2.8×10^7	Between 0.50 and 0.6 c.c.
8.4×10^{10}	"	4.1×10^8	" 0.75 " 1.00 "
1.0×10^{10}	Scant	1.0×10^8	" 1.5 " 2.0 "
6.5×10^7	Very scant	7.5×10^{10}	More than 2.0 c.c.

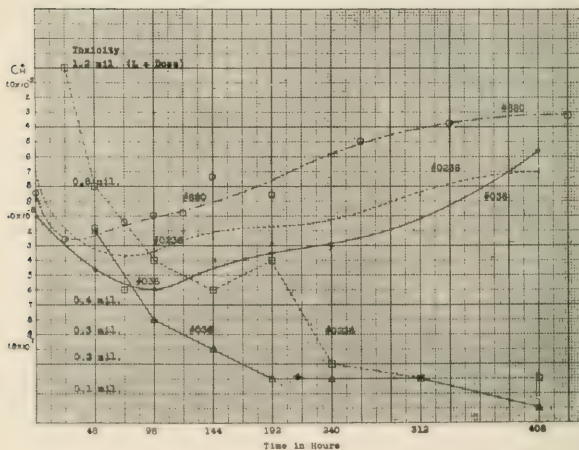


Fig. 2

Inspection of the data tabulated in Table I shows that the optimal zone for metabolic activity of Bact. diphtherie is in the alkaline region. While good growth of the organism appears to

be possible within hydrogen-ion concentration limits ranging from about $C_H^+ = 1.0 \times 10^{-6}$ to about $C_H^+ = 8.4 \times 10^{-10}$, maximum production of toxin seems to occur only where the reaction of the bouillon falls in a concentration range from about $C_H^+ = 7.0 \times 10^{-8}$ to about $C_H^+ = 5.0 \times 10^{-9}$. Antiseptic action, as evidenced by scant growth, is apparently exerted on the acid side around $C_H^+ = 6.5 \times 10^{-6}$, while on the alkaline side it does not come into consideration until about $C_H^+ = 5.3 \times 10^{-10}$ is reached.

With the foregoing results at hand, experimentation was now undertaken to determine the changes in reaction and toxin production which take place in plain bouillon during the growth of Bact. diphtheriæ cultures under optimum conditions. In addition to the Park strain (No. 036) employed above for the antiseptic value test, another toxicogenic strain (No. 0236) originally obtained from McFarland, and an avirulent nontoxicogenic strain (No. 880) isolated by H. C. Ward of this laboratory were studied. The preparation of the bouillon for this purpose and the inoculation technic have been detailed in the preceding section. The accompanying curves shown in Fig. 2 have been plotted using the data obtained with the three cultures, and tabulated in Table II.

TABLE II

CHANGES IN H-ION CONCENTRATION AND TOXICOGENICITY DURING GROWTH OF BACT. DIPHTHERIÆ IN BOUILLON.

(Park) Strain No. 036			(McFarland) Strain No. 0236			(Avirulent) Strain No. 880		
TIME IN HOURS	C_H^+	TOXICITY L+DOSE	TIME IN HOURS	C_H^+	TOXICITY L+DOSE	TIME IN HOURS	C_H^+	TOXICITY M.F DOSE
0	1.0×10^{-5}	—	0	7.0×10^{-9}	—	0	8.5×10^{-9}	—
48	4.6×10^{-8}	0.65 c.c.	24	2.1×10^{-8}	1.0 c.c.	24	3.3×10^{-8}	No reaction
			48	3.2×10^{-8}	0.8 "	48	1.9×10^{-8}	2 c.c.
			72	3.8×10^{-8}	0.45 "	72	1.3×10^{-8}	
96	6.0×10^{-8}	0.35 "	96	3.4×10^{-8}	0.55 "	96	1.1×10^{-8}	
			120	2.1×10^{-8}	0.35 "	120	9.5×10^{-9}	No reaction
144	4.0×10^{-8}	0.25 "	144	2.5×10^{-8}	0.45 "	144	7.4×10^{-9}	2 c.c.
192	2.9×10^{-8}	0.15 "	192	2.3×10^{-8}	0.55 "	192	8.3×10^{-9}	
240	3.0×10^{-8}	0.15 "	240	1.6×10^{-8}	0.15 "			
						264	5.5×10^{-9}	
312	1.2×10^{-8}	0.15 "	312	8.7×10^{-9}	0.15 "	336	3.8×10^{-9}	Necrosis 2 c.c.
408	4.7×10^{-9}	0.05 "	408	7.0×10^{-9}	0.15 "	432	3.2×10^{-9}	Necrosis 2 c.c.
			528	5.0×10^{-9}	0.15 "			

Comparison of the hydrogen-ion concentration curves of Fig. 2 shows a decided similarity with all three of the strains

examined. There is, at first, a small production of acid, as shown by an increase in the concentration of hydrogen ions, which soon reaches a maximum, the amount of acid produced varying with the organism. The Park strain (No. 036) gives the maximum amount of acid, the other toxicogenic strain (McFarland No. 0236) produces nearly as much, while the avirulent strain develops only about one-half of the amount.

Inspection of Fig. 2 indicates that the slope of the acid production curves is very nearly the same for the three strains, so that the total increase in hydrogen-ion concentration directly depends on the length of time the organism undergoes acid fermentation. Strain No. 036 required four days to reach a maximum value, No. 0236 apparently had attained this point in 72 hours while No. 880 seems to have begun an alkaline fermentation after the first day. In fact, it will be noted that all three strains show a reversal, followed by alkaline fermentation which apparently continues until an antiseptic hydrogen-ion concentration is reached.

By way of obtaining some information as to the source of the acid production in plain bouillon, two sets of media were prepared as already directed. One set consisted of the beef infusion used in the preceding experimentation with 0.5 per cent sodium chloride, and the other had 2 per cent of the peptone and 0.5 per cent of the sodium chloride. Both sets were inoculated with starters of the Park strain (No. 036), and the hydrogen-ion concentration changes occurring in each during growth were now studied in accordance with the technic already employed. The results are shown in Table III.

TABLE III
HYDROGEN-ION CONCENTRATION CHANGES DURING GROWTH OF BACT. DIPHTHERIE IN STRAIGHT BEEF INFUSION AND 2% PEPTONE SOLUTION

<i>Beef Infusion</i>			<i>2% Peptone Solution</i>		
TIME IN HOURS	ϵ H	TOXICITY M F DOSE	TIME IN HOURS	ϵ H	TOXICITY M F DOSE
0	8.5×10^{-9}		0	7.5×10^{-9}	
24	9.4×10^{-9}	No reaction	24	8.4×10^{-9}	No reaction
48	1.2×10^{-8}	" "	48	1.0×10^{-8}	2 c.c.
96	2.7×10^{-8}	" "	96	8.7×10^{-9}	" "
168	2.7×10^{-8}	" "	168	7.0×10^{-9}	Slight reaction
240	1.6×10^{-8}	" "	240	6.5×10^{-9}	2 c.c.
408	5.9×10^{-8}	" "	408	6.1×10^{-9}	No reaction
					2 c.c.

It is readily apparent from the above data that both components of plain bouillon permit of acid fermentation by *Bact. diphtheriæ*. As was expected, the growth in straight infusion was scant, while that in the 2 per cent peptone solution (+ salt) appeared only about half as heavy as in regular bouillon. Table III shows that in plain beef infusion, the diphtheria bacillus produces a small amount of acid which reaches a maximum in about four days, after which the hydrogen-ion concentration remains practically constant. In the peptone solution, there is also an initial acid production increasing to about the third day, but then followed by a reversal and steady decrease in hydrogen-ion concentration as in bouillon. Estimation of the amounts of acid produced in each case indicates that the sum total of both sets very nearly approximates the initial H-ion increase during growth in plain bouillon, as given in Fig. 2.

The toxicity values for No. 036 show a steady increase until the eighth day. Five days later the value appears to be constant, but increases in toxicity at the end of seventeen days. The toxin development in the case of No. 0236 is irregular, fluctuating up to the eighth day, after which there is a rapid increase in toxicity, reaching a maximum on the thirteenth day and remaining constant at this value to the end of the experiment. It is interesting to note that the avirulent strain (No. 880) produces no toxin at first (2 c.c. dose), but sufficient is developed at the end of two weeks to cause necrosis at the site of injection with the same dose. That it is actually diphtheria toxin which is produced, is shown by the fact that addition of antitoxin (as in testing by L⁺ dose) causes neutralization and no necrosis.

DISCUSSION.

As has been found true in the case of several other organisms, notably in the colon and streptococcus groups (Michaelis and Marcora,¹⁵ Ayers,¹⁶ Itano¹⁷), the hydrogen-ion concentration of the medium, from the data presented above, is seen to have a decided influence on the metabolism of *Bact. diphtheriæ*. While there is relatively a wide range, from about $C_H^+ = 1.0 \times 10^{-3}$ to about $C_H^+ = 8.4 \times 10^{-10}$, in which good growth can take place, maximal elaboration of toxin occurs only in a small zone of hydrogen-ion concentration, covered by $C_H^+ = 7.0 \times 10^{-8}$ to

$C_H = 5.0 \times 10^{-7}$. The upper limits of alkalinity very closely approximate in value those obtained by the author for the H-ion concentration of antidiphtheric serum (equine) and the lower is very near the C_H^- value given by McClendon and Magoon¹⁸ for blood.

It is interesting to note from Table I that *Bact. diphtheriæ* can tolerate a much greater concentration of hydroxyl ions (stronger alkaline reaction) than acid ions before a value inhibitory to growth is encountered. In all probability, the antiseptic C_H^+ values obtained with the Park strain (6.5×10^{-6} and 5.3×10^{-10}) are of general application to *Bact. diphtheriæ* since "starters" of the McFarland Strain (No. 0236) fail to develop at $C_H^- = 6.5 \times 10^{-6}$ and give only very scant growth in a medium reading $C_H^+ = 5.0 \times 10^{-10}$. No growth at all can be obtained with the avirulent strain at either of the above mentioned hydrogen-ion concentrations.

Confirming, in a general way, the results obtained by other investigators, the curves plotted in Fig. 2 show that *B. diphtheriæ*, when cultivated in plain bouillon undergoes an initial acid fermentation. Contrary to what might be supposed from the introductory review, the total increase in hydrogen-ion concentration, even with the most vigorous strain (Park, No. 036) is relatively small (from about $C_H^+ = 1.0 \times 10^{-8}$ to $C_H^+ = 6.0 \times 10^{-8}$), and appears to be due to an acid fermentation of some constituent, very likely carbohydrate in nature, in both the beef infusion and the peptone. It should also be noted that even at the point of maximum acid development, the reaction is definitely alkaline.

The results given in Table I strongly indicate that an actual acid reaction ($C_H^+ = 1.0 \times 10^{-7}$ = neutral) has a destructive action on diphtheria toxin. From the amount of acid produced by both of the above toxicogenic strains, it would seem that where the initial C_H^+ of the medium is appreciably greater than 1.0×10^{-8} , the increase in hydrogen-ion concentration resulting from growth would be sufficient to produce a final acid reaction destructive to the toxin. This is actually found to be the case. Table I shows that where the initial reaction of the bouillon is $C_H^+ = 1.0 \times 10^{-8}$, a strong toxin with L₅₀ dose of less than 0.25 c.c. is obtained. On the other hand, a slight increase in the initial reaction to

$C_H^+ = 1.0 \times 10^{-7}$ is sufficient to make the final potency drop to an L^+ dose of about 0.7 c.c.

The total amount of acid produced appears to be a specific property of each individual strain. The avirulent strain develops only about half as much acid as do the toxicogenic strains, the increase in hydrogen-ion concentration reaching a maximum soon after the first day in the former case. It may be probable that this diminished capacity for acid fermentation is a general property of avirulent strains and is possibly an index of decreased metabolic activity.

The data given in Tables II and III further show that the natural course pursued by *Bact. diphtheriae* growing in plain bouillon is in the direction of increased alkali production. This decrease in hydrogen-ion concentration takes place steadily after the initial acid fermentation and reversal, and apparently continues until a limiting concentration is attained.

It is obvious from the curves plotted in Fig. II that in the normal development of *Bact. diphtheriae* in bouillon, the organism may produce the same hydrogen-ion concentration at two different intervals, which represent wide variations in potency. Other than the fact that it is necessary to have the initial reaction of the bouillon within the alkaline limits above mentioned, and that potent toxin will have an alkaline reaction, the experimental data indicate that there is no direct relationship during growth between the hydrogen-ion concentration of the medium and the production of toxin.

In conclusion, I desire to express my most sincere thanks to Professor F. E. Bartell of the University of Michigan for valued advice on the determination of hydrogen-ion concentration.

CONCLUSIONS.

1. Toxin of maximum potency is produced in bouillon by *Bact. diphtheriae* only when the initial reaction falls within a certain zone of alkalinity, included within the hydrogen-ion concentration limits of about 7.0×10^{-8} to about 5.0×10^{-9} . Luxuriant growth of the organism appears to be possible where the reaction of the bouillon ranges from about $C_H^+ = 1.0 \times 10^{-7}$ to about $C_H^+ = 8.4 \times 10^{-10}$.

2. When cultivated in plain bouillon under optimal conditions, *Bact. diphtheriæ* undergoes an initial increase in hydrogen-ion concentration. This is soon followed by a steady decrease until apparently a limiting alkaline reaction is attained. The total acid produced is relatively small and seems to vary in amount with each individual strain. Toxicogenic strains appeared to develop more acid than an avirulent strain. The initial increase in hydrogen-ions is due to fermentation of some constituent in both peptone and beef infusion.

3. No direct relationship can be found between the hydrogen-ion concentration of the medium and toxicity during the growth of *Bact. diphtheriæ*.

BIBLIOGRAPHY

- ¹Roux and Yersin: *Ann. de l'Inst. Pasteur*, 1888, 1889, 1890, 1894.
- ²Spronck: *Ann. de l'Inst. Pasteur*, 1895, ix, 758; *Ibid.*, 1898, xii, 700.
- ³Park and Williams: *Jour. Exper. Med.*, 1896, i, 164.
- ⁴Madsen: *Ztschr. f. Hyg. u. Infektionskrankh.*, 1897, xxvi, 157.
- ⁵Smith: *Jour. Exper. Med.*, 1899, iv, 333.
- ⁶Hitchens: *Jour. Med. Research*, 1904-5, xiii, 523.
- ⁷Lubenant: *Arch. f. Hyg.*, 1908, lxvi, 305.
- ⁸Jacobsen: *Centralbl. f. Bakteriöl.*, 1910-11, lvii, Part 1, p. 16.
- ⁹Clark: *Jour. Infect. Dis.*, 1915, xvii, 129.
- ¹⁰Davis: *Jour. Lab. and Clin. Med.*, 1917, iii, No. 2, p. 75.
- ¹¹Clark and Lubs: *Jour. Bact.*, 1917, ii, Nos. 1, 2, 3, pp. 1, 109, 191.
- ¹²Hurwitz, Meyer and Ostenberg: *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 17.
- ¹³Bovie: *Jour. Med. Research*, 1915, xxxiii, 295.
- ¹⁴Bartlett: *Jour. Am. Chem. Soc.*, 1917, xxxix, No. 4, p. 630.
- ¹⁵Michaëlis and Marcora: *Zeitschr. f. Immunitätsforsch. Exper. Ther.*, 1912, Orig., xiv, 170.
- ¹⁶Ayers: *Jour. Bact.*, 1916, i, 84.
- ¹⁷Itano: *Bull. Mass. Agr. Exper. Sta.*, 1916, No. 167.
- ¹⁸McClendon and Magoon: *Jour. Biol. Chem.*, 1916, xxv, No. 3, p. 669.

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**INVESTIGATIONS ON THE COMPOSITION OF OIL OF
CHENOPODIUM AND THE ANTHELMINTIC
VALUE OF SOME OF ITS COMPONENTS.**

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Oil of chenopodium is an unusually valuable and potent anthelmintic, but like practically all potent anthelmintics it exerts an injurious action on the host animal to some extent, an action which is usually very slight, but sometimes severe. This undesirable effect has been noted by Rose¹ in the following terms:

Alarming symptoms, and sometimes death, have been reported in the Southern States, the West Indian colonies, Panama, Nicaragua, Ceylon, and Egypt following the administration of the drug in accordance with accepted methods of treatment, and in nearly every instance in less than the maximum dose. Extreme caution in the use of the drug is therefore indicated until its proper method of preparation has been learned, its chemical composition and stability standardized, and a safe dosage and method of administration established.

As one of us (Hall²) has noted elsewhere, the injury to a patient probably depends partly on the rate of absorption of the anthelmintic, and in this connection there are two factors to consider, one the immediate local irritant effect of rapid absorption on the digestive tract, and the other the more remote irritant and toxic systemic effects on the circulatory, respiratory, excretory and nervous systems.

Of these two factors, the last-named, the systemic effect, has received some little attention from a number of workers, especially Salant and his collaborators^{3, 4, 5, 6} who have noted the depressant or irritant effects on the circulatory, respiratory, and renal mechanisms, and on the unstriated musculature of the intestine. Zeigler⁷ has in a general way confirmed their work.

As regards the other factor, the irritant effect of chenopodium on the digestive tract, little is said, though Brüning⁸ noted in

1906 the production of gastro-intestinal hyperemia and petechiæ by chenopodium. The casual statements of various writers who have noted vomiting or nausea show that there is an inkling, but vaguely formulated as yet among members of the medical profession, to the effect that oil of chenopodium acts at times as a gastro-intestinal irritant. Postmortem findings on experiment dogs in this laboratory show that oil of chenopodium does exert this irritant action to varying degrees in different individuals. This paper deals specifically with this point, gastro-intestinal irritation, and indicates what appears to be the particular cause for the irritation and the remedy for it.

In a paper by Hall and Foster¹⁰ covering work done in the United States Bureau of Animal Industry, there are detailed some experiments in which oil of chenopodium was administered in connection with liquid petrolatum. Some similar experiments are noted here. These experiments show that the combination is not a success; the anthelmintic efficacy of the drug is seriously impaired and occasional dogs show injurious effects. These findings deserve emphasis, for the reason that the idea of combining oil of chenopodium with liquid petrolatum has occurred to others as being perhaps a valuable combination. Thus Hoelscher¹¹ in a discussion of mineral oil as a vehicle says:

Particularly impressive is the possibility of overcoming hookworm and other intestinal parasites when the oil is combined with chenopodium, iodine, oil of turpentine, guaiacol, thymol, pelletierine, etc., and since the oil is not absorbed one may assume that these rather toxic agents may also pass through the gastro-intestinal tract unchanged. This, however, calls for considerable investigation.

Vanderkleed¹² has noted the finding, in a market sample of oil of chenopodium, of adulteration with 44.3 per cent of an odorless fixed oil, which might have been mineral oil. Additional evidence obtained in this laboratory as to the loss of potency of oil of chenopodium when combined with liquid petrolatum led to some experiments by one of us (Hamilton) to ascertain the solubility of chenopodium in liquid petrolatum. It was observed that when oil of chenopodium was first shaken up with liquid petrolatum, the resulting preparation was slightly opaque. When allowed to stand, a small amount of a thick, dark-brown material separated out at the bottom, the remainder of the preparation

becoming clear and light-colored. An investigation of the anthelmintic properties of these components led to an investigation of the distillation products of oil of chenopodium, with results which may be summarized about as follows:

1. Distillation experiments indicate that oil of chenopodium, like most of the volatile oils, has no distinct boiling point, being composed of a mixture of constituents of undetermined character. At low mercury pressures the oil distills steadily over a wide range of temperatures, leaving a residue amounting to about 5 per cent which is only partially volatile and is gummy or resinous in character.

The first distillate is very light colored, in some cases almost water white. The distillate becomes progressively darker as it distills at higher temperatures and soon acquires nearly the color of the original oil. The odor of the most volatile portion most nearly resembles that of the original oil, while the last distillate and that remaining in the flask has an odor resembling turpentine.

The steadily rising temperature and equally steady distillation of the oil gave no line of demarcation to indicate that any considerable quantity of a single constituent is present.

These results are distinctly at variance with those reported by Schimmel and Company¹² and by Nelson¹³, both of whom separated from oil of chenopodium a considerable portion with a distinct boiling point, to which portion the name ascaridol was given.

2. Tests of the distillation products obtained indicate that the greatest anthelmintic efficacy resides in the lightest fraction of the oil, the efficacy suffering a diminution as the heavier fractions are used. Furthermore, these tests indicate that the heavier fractions have a well-marked action as gastro-intestinal irritants, capable of producing, in doses equivalent to the therapeutic dose of the oil, hemorrhagic conditions of the stomach and intestine, even in the presence of castor oil, which is highly protective. It would therefore appear that if the findings reported here are confirmed, the product marketed as oil of chenopodium should be redistilled to eliminate a fraction which has less anthelmintic value and more irritant and toxic properties than the lighter fraction. Such a procedure should add to the value and safety of this drug.

The detailed experiments performed by each of us are given below.

INVESTIGATIONS ON THE COMPOSITION OF OIL OF
CHENOPODIUM.

HERBERT C. HAMILTON.

The investigation of the chemical and physical characteristics of this oil was suggested by the peculiar results obtained when it was mixed with petroleum oil and administered to dogs. The severe effects on some dogs and the failure of the oil of chenopodium to produce its typical anthelmintic action led the writer to believe that the active constituent was soluble in the petroleum oil while a toxic and irritating constituent was insoluble and was precipitated on the stomach and intestinal walls, where its absorption was unhindered and therefore prompt. While this may be partly true in the light of our further investigations, the point is of little importance because of the fact that the petroleum oil is not an active purgative and the mixture has no practical value.

These preliminary experiments suggested further work with the idea of determining whether any single fraction of the oil has the anthelmintic property and whether it is possible to eliminate the constituents responsible for the toxic and irritating effects occasionally experienced in clinical work.

The first recorded chemical investigation of this oil is that of Kremers,¹⁴ who, in attempting to distill it at atmospheric pressure, found that decomposition of an explosive character occurred when the oil was heated to boiling temperature.

This work was followed up by Schimmel and Company¹² on some oil specially prepared by themselves from the seed. They verified Kremers' work in finding that above 100° C. at atmospheric pressure a decomposition of extreme violence takes place, making it necessary to carry out further experiments at reduced pressure. At 5 mm. pressure they found that the oil began to distill at 53° C., 25 per cent coming over at 53° to 79°. At 83° (4 to 5 mm. pressure) they obtained a fraction which constituted the principal part of the oil and to which they gave the name ascaridol. Its optical rotation was found to be -4.2° . It was described as having an offensive odor but its chemical character was not determined. In this particular sample of oil they found 20 per cent hydrocarbons, and 65 to 70 per cent of the fraction named ascaridol. The proportions were found to vary in different lots of the oil.

Nelson¹⁵ working with a sample probably one year old found the original oil to have a rotation of -0.35° .^{*} Fractionating at 8 mm. he obtained 15 per cent of a hydrocarbon—the first distillate—and 70 per cent of the substance to which Schimmel gave the name ascaridol. When purified its boiling point was 96° to 97° C. at 8 mm. pressure with an optical rotation of $+0.7^{\circ}$.

Nelson states:

Schimmel and Company found ascaridol to be inactive. The slight optical activity of ascaridol obtained from this sample is no doubt due to the rather large amount of camphor present, from which the ascaridol was not entirely freed on fractionation.

A careful reading of their report fails to verify the accuracy of the statement, since Schimmel and Company gave the optical rotation as -4.2° . This seems the more probable value since none of the fractions obtained in my experiments was found to have a plus rotation or to be inactive. The product found by Schimmel and Company to be optically inactive was not ascaridol, but a conversion product of the same formula having entirely different properties.

In the following experiments in this laboratory, an attempt was made to obtain ascaridol by fractionation.

Distillation at 25 to 30 mm. of mercury, the lowest pressure obtainable at the time, showed that at no single point was any considerable proportion of the oil volatile. It began to distill at approximately 85° C. and continued to come over at a practically uniform rate through a range of temperatures up to 140° C.

The distillation was carried out in a Ladenburg distilling flask with the bulb of the thermometer about 1 inch below the outflow tube. The following percentages were obtained at approximately the temperatures in the parallel column:

TEMPERATURE C.	YIELD
	<i>per cent</i>
85° – 100°	20
100° – 110°	25
110° – 125°	30
125° – 140°	20
above 140°	5

^{*}This optical rotation does not conform to the U. S. P. requirements for the genuine oil.

The difficulties met with in maintaining constant temperatures and pressure after removing the distilled fraction, make these figures unreliable except approximately.

While these results seemed to disprove the statement that any considerable proportion of oil of chenopodium has a single boiling point, the difference between the pressure at which the above distillation was carried out and that employed by Nelson suggested the use of a more efficient vacuum pump. Under a vacuum of 2 to 5 mm. of mercury no marked difference was noted except that there was a shift of about 40° in the boiling points of the fractions. Distillation began at about 50° and the oil was completely distilled at 100°, except a gummy residue amounting to about 5 per cent. Redistillation of the various portions showed practically no evidence of a material change in the boiling points.

The different fractions were found to be different in anthelmintic activity. The difference is sufficiently marked to show that the fraction having the lowest boiling point and highest optical rotation is the most efficient and that this anthelmintic efficiency decreases as the distillation temperatures of the different fractions increase.

Examination of the oils for optical activity showed the following results:

FRACTION	ROTATION
Original oil	— 6.2°
85°–100°C.	— 13.9
100°–110°C.	— 6.6°
110°–145°C.	— 1.2°
85°–125°C.	— 7.7°
Not distilled at 125°	— 1.1°

A sample of Schimmel and Company's oil was distilled at 25 mm. pressure and the following data obtained:

BOILING POINTS OF FRACTION	YIELD per cent	OPTICAL ROTATION	SPECIFIC GRAVITY
Original oil		— 6.0°	0.957
85°–100°C.	24	— 13.4°	0.901
100°–110°C.	22	— 7.3°	0.933
110°–120°C.	26	— 2.6	0.993
Above 120°	26	— 1.5	1.030

Residue not distilled amounted to about 6 to 8 per cent, but on account of the small amount and gummy properties no examination was made of it.

In the course of the experiments a number of different parts of oil of chenopodium or treated samples were prepared by one of us (Hamilton) and tested for anthelmintic action by the other (Hall).

No. 1. The part insoluble in medicinal petroleum oil (American Oil).

No. 2. The part entirely soluble in this oil but recovered, being separated by shaking out with alcohol and the alcohol removed by evaporation.

No. 3. A sample consisting of oil of chenopodium dissolved in American Oil.

No. 4. A sample of oil of chenopodium treated with alcoholic caustic potash to saponify it. The mixture was shaken thoroughly with water to remove water soluble constituents, and the supernatant layer separated for testing.

No. 5. Distillate at 30 mm. mercury, including those portions boiling below 125°C. Approximately 60 per cent. This was a preliminary attempt, imperfectly carried out.

No. 6. Second portion from above distillation, including those fractions between 125° and 140°C. 33 per cent.

No. 7. Residue not distilled at 140°C. 30 mm. mercury.

No. 8. Residue remaining after evaporation of oil of chenopodium on the steam bath. About 3 per cent.

A second series of distillates were obtained by more careful fractionation.

No. 9. First distillate including fractions boiling below 100°C. at 30 mm. mercury. 26 per cent.

No. 10. Second distillate, 100° to 110°C. 28 per cent.

No. 11. Third distillate, 110° to 140°C. 30 per cent.

No. 12. Residue not distilled at 140°C.

A third series of distillates.

No. 13. Total distillate to 125°C. 74 per cent.

No. 14. Residue not distilled at 125°C. 26 per cent.

No. 15. Oil of chenopodium, Schimmel, distilled to obtain all that portion volatile below 130°. About 95 per cent distilled.

No. 16. Residue from above. 5 per cent.

No. 17. Sample 9 treated with freshly prepared FeSO_4 .

No. 18. Similar to No. 15 but distilled at 2 to 5 mm. mercury. All but about 6 per cent distilled below 100°C.

No. 19. Residue from above.

Samples 13 and 14 were prepared to obtain as nearly as possible the active but less irritating part in the first distillate. Sample 15 was prepared to determine whether the reduced oil—the glycol described by Nelson¹²—has acquired different properties.

In addition to the above experiments some blood-pressure tests were applied to the original oil and to two of the fractions to determine whether the characteristic depressant effects described by Salant and Livingston¹³ could be used as a means of selecting certain portions less depressant. These were carried out with emulsions of the oil prepared in various ways, the amount used being 0.03 mil of the oil. The dogs were anesthetized with trichlor-tertiarybutyl alcohol (chloretone) and the blood-pressure recorded by mercury manometer. The depressant effect followed promptly, showing considerable fall. This effect, however, seemed to have no great degree of regularity, either in different dogs or on the same dog.

The first distillate and the undistilled residue were then compared, with the result that while this depressant effect is found to be inconstant and consequently of little value for comparing different samples, nevertheless no material difference could be observed between the two fractions which represent the lowest and the highest boiling parts of the oil.

The failure to obtain a material quantity of the oil having a fixed boiling point cannot be explained at this time and is merely recorded without comment. The results of the above experiments would indicate that the so-called ascaridol should have been found near the end of the distillation both from the recorded boiling point—96° to 97° C. at 8 mm. mercury—and from the observed low optical activity.

Data on this point in the work reviewed are insufficient to determine exactly what position in the whole oil was occupied by the 70 per cent which appeared to be a chemical compound of definite composition. Without any question, however, it did not include the first fraction.

Our observations showed that the most desirable part of the oil and that having the characteristic odor and effect of the original oil is that part containing the lower boiling fractions and having the highest optical activity.

THE ANTHELMINTIC VALUE OF CHENOPODIUM COMPONENTS.

MAURICE C. HALL.

Inasmuch as chenopodium is specifically ascaricidal, having a decidedly higher efficacy, when administered in a single therapeutic dose, against ascarids than is shown by any other known anthelmintic, similarly administered, against any worm, the common ascarid of the dog was used as the test worm in determining the anthelmintic value of chenopodium products. The writer¹⁶ has elsewhere pointed out that anthelmintics, or other substances, passing the ileocecal or ileocolic valve do not always enter the cecum, so that the whipworm, located usually in the cecum, will not serve as a test worm and is disregarded here. Hookworms cannot be removed with anything like 100 per cent efficacy by safe therapeutic doses of any anthelmintic that we are at present aware of, so these worms are disregarded here. The results of numerous tests by the writer¹⁷ having established that for oil of chenopodium a dose of 0.1 mil per kilo can be depended on to remove all ascarids from the dog in the great majority of cases, this dose is referred to in this paper as the standard dose. The lesions in the gastro-intestinal tract were determined by the slitting of the entire tract, with an enterotome, from the stomach through the rectum, and the careful washing and examination of the mucosa.

To study the action of oil of chenopodium in a certain limited range of administration, such as its action with liquid petrolatum, and to ascertain the efficacy of the various chenopodium preparations furnished by my colleague (Hamilton), the following experiments were performed, the method used being that of Hall and Foster¹⁸—*i.e.*, the collection of all worms from the feces after treatment and from the dog postmortem:

Dog 26, a terrier weighing 5.5 kilos, was given 10 minims of oil of chenopodium, a dose slightly exceeding the standard 0.1 mil per kilo dose, in a soluble elastic capsule, followed immediately by 30 mls of liquid petrolatum (American oil). The dog passed 11 ascarids in the next 4 days. The animal was killed the fourth day and found to have 6 ascarids in the stomach and 17 in the small intestine. The treatment was therefore 35 per cent effective. The small intestine showed locally inflamed areas throughout. This illustrates the diminished efficacy of chenopodium when given with liquid petrolatum.

Dog 121, a mongrel weighing 12.75 kilos, was given 2.5 mls of oil of American wormseed (a dose rate of 0.2 mil per kilo, or double that which has been reported by Hall¹⁷ as necessary for oil of chenopodium when given with castor oil) shaken up with 30 mls of liquid petrolatum. Between 5 and 10 mls of this material was lost in dosing, leaving an amount usually more than sufficient to attain 100 per cent efficacy against ascarids if given with castor oil. No feces were passed the day after treatment, indicating that the mineral oil did not overcome the constipating effect of the chenopodium. No worms were passed during the 4 days after treatment and postmortem on the fourth day showed 28 whipworms present. The treatment was therefore 0 per cent effective against whipworms, which is not surprising, as single dose treatments are commonly ineffective against these worms. The digestive tract was normal. The experiment was inconclusive.

Dog 247, a mongrel bulldog weighing 10 kilos, was given oil of chenopodium at the rate of 1 mil per kilo, which is double the rate regarded by Zeigler⁷ as lethal for dogs, thoroughly mixed with 50 mls of liquid petrolatum. (Salant and Nelson⁸ did not find approximately 0.5 mil per kilo lethal for dogs.) Part of the dose evidently entered the lungs. The dog vomited within twenty minutes and defecated within twenty-five minutes. The next day the dog was curled up in its cage, much depressed, trembling, and with its eyes shut. The temperature was 101.2°. The next day the dog had passed 6 ascarids. The dog was now comatose, the temperature dropped to 94.7°, breathing ceased, the heart beat a few times at long intervals and then stopped, except for a slow peristalsis-like movement visible on opening the chest and which persisted even when the heart was cold. No worms were found postmortem, so the treatment with this very large dose of chenopodium was 100 per cent effective against the test worms, ascarids, even when masked with liquid petrolatum. All the left lobes of the lung were in a state of red hepatization, probably from the drench. The kidneys were highly inflamed. The stomach was hemorrhagic in places and the tops of the gastric folds were necrotic in places. The Peyer's patches showed hemorrhages in the mucosa or submucosa, and there were areas in the jejunum that appeared eroded. The large intestine and cecum were inflamed and in places hemorrhagic. The dose of oil of chenopodium was so large that the liquid petrolatum did not sufficiently mask its anthelmintic activity or its irritant action.

Preparation 1, the part of oil of chenopodium insoluble in liquid petrolatum, was tested as follows:

Dog 136, a mongrel weighing 8.5 kilos, was given preparation 1 at the rate of 0.04 gram per kilo, a dose of 0.34 gram, followed immediately by 30 mls of castor oil. No worms were passed. The dog was killed the fourth day after treatment, and no worms recovered postmortem. The digestive tract was normal.

Dog 142, a bulldog weighing 12.5 kilos, was given preparation 1 at the rate of 0.02 gram per kilo, a dose of 0.25 gram, followed immediately by 30 mils of castor oil. The second day after treatment the dog passed 1 ascarid. The dog was killed the fourth day after treatment and on post-mortem had 3 whipworms. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against whipworms. There was a group of fine petechiæ in the duodenum and there were 2 small inflamed areas in the jejunum.

Dog 144, a mongrel weighing 14.5 kilos, was given preparation 1 at a rate slightly less than 0.014 mil per kilo, for a total of 0.2 gram, followed immediately by 30 mils of castor oil. The dog passed no worms. The animal was killed the sixth day after treatment and on postmortem was found to have 1 ascarid, 7 whipworms, 2 *Tænia pisiformis* and 2 *Dipylidium*. The treatment was therefore 0 per cent effective against ascarids, whipworms, *Tænia* and *Dipylidium*, which is not surprising in view of the fact that this is a light dose. Such a dose should only be effective against the test worms, ascarids, in case the anthelmintic value of oil of chenopodium resided in this preparation, with the effective chenopodium dose 0.1 gram per kilo, but our other experiments show that the anthelmintic properties of oil of chenopodium are well distributed through the oil, the lighter, rather than the heavier, parts, excelling in this respect. The digestive tract in this dog showed numerous small inflamed points apparently corresponding to inflamed villi on close examination, sometimes alone and at times aggregated to form inflamed areas, throughout the small intestine.

Preparation 2, the recovered part of the oil of chenopodium which is soluble in liquid petrolatum, was tested as follows:

Dog 147, a mongrel weighing 7 kilos, was given this preparation at the rate of 0.2 mil per kilo, a dose of 1.4 mils. The next day the dog passed 33 ascarids. The animal was killed the fourth day and found free from worms. The treatment was, therefore, 100 per cent effective against the test worms, ascarids. The lower portion of the ileum was moderately inflamed. The portion of the oil of chenopodium which dissolves in liquid petrolatum is therefore anthelmintic.

Preparation 3, the petrolatum-soluble part of oil of chenopodium left dissolved in liquid petrolatum, was tested as follows:

Dog 132, an Airedale weighing 18.25 kilos, was given this preparation containing oil of chenopodium at the rate of 0.2 mil per kilo, or 3.65 mils, dissolved in 26.35 mils of liquid petrolatum to make a total of 30 mils. The dog passed no worms and was killed the fourth day. No worms were found postmortem. The individual villi over large areas in the duodenum and upper jejunum were a pronounced yellow; the remainder of the digestive tract was normal.

Dog 140, a mongrel weighing 6.75 kilos, was given this preparation containing oil of chenopodium at the rate of almost 0.7 mil per kilo, a dose of 4.6 mls, dissolved in 23.4 mls of liquid petrolatum to make a total of 30 mls. The dose used is in excess of the minimum lethal dose of 0.5 mil per kilo, as ascertained by Zeigler.⁷ The dog passed no worms and was killed the fourth day. On postmortem, the animal was found to have 5 ascarids and 4 tapeworms. The treatment, in spite of the excessive dose employed, was therefore 0 per cent effective against the test worms, ascarids, and against tapeworms, indicating that the anthelmintic properties of the drug, shown to be highly active in the experiment with preparation 2 on dog 147, were effectively masked by the liquid petrolatum. The digestive tract showed 3 small hemorrhages in the lower ileum.

The above experiments, while not entirely satisfactory, seemed to show the following facts: Oil of chenopodium dissolves to a large extent in liquid petrolatum, leaving a portion amounting to about 10 per cent, a dark, gummy substance, that does not dissolve but settles out at the bottom; both the lighter soluble part and the heavier insoluble part are anthelmintic; when the soluble part is administered dissolved in an excess of liquid petrolatum the anthelmintic efficacy is masked, so much so that a dose of 0.7 mil per kilo, more than a minimum lethal dose, dissolved in five times its volume of liquid petrolatum, has an anthelmintic efficacy of zero, and exerts almost no bad effect on the digestive tract; when oil of chenopodium is given in double the minimum lethal dose, dissolved in five times its volume of liquid petrolatum, so far as it is soluble in this, death ensues inside of forty-eight hours, due in part, probably, to the irritant effects of the approximate 0.1 mil per kilo of insoluble material present and in part to the large dose of oil taken in.

The chief value of the above experiments was in suggesting additional experimentation. Two products formed by combination with oil of chenopodium were prepared by Hamilton and tested by me. Preparation 4, made by treating oil of chenopodium with KOH, was tested as follows:

Dog 149, a spaniel mongrel weighing 9.25 kilos, was given this preparation at the rate of 0.2 mil per kilo, for a dose of 1.9 mls, followed immediately by 30 mls of castor oil. The next day the dog passed 1 hookworm. The dog was killed on the sixth day and on postmortem was found free from worms. The treatment was therefore 100 per cent effective against hookworms. The digestive tract was normal.

Dog 160, a mongrel weighing 14.5 kilos, was given this preparation, No. 4, at the rate of 0.2 mil per kilo, for a dose of 2.9 mils, without castor oil. The animal had a severe diarrhea at the time of treatment. It vomited twice soon after treatment and passed 17 ascarids in about a half hour. The next day the dog was very weak and trembling and was given 30 mils of castor oil about twenty hours after treatment. The second day after treatment, the animal was entirely paralyzed, dying during the day. No worms were found postmortem, so the treatment was 100 per cent effective against the test worms, ascarids. The greater curvature of the stomach was somewhat inflamed and the remainder of the digestive tract was inflamed and hemorrhagic. It is likely that there was an enteritis, indicated by the diarrhea, at the time of treatment, and that this was aggravated to a fatal termination by the treatment, especially in the absence of the protection of castor oil, which was administered too late to be of value. The KOH may form a more rapidly soluble combination with the oil of chenopodium.

The conclusion from the above is that the treating of oil of chenopodium with KOH does not deprive it of its anthelmintic properties, but may increase its injurious effects on the host. As the preparation did not appear to us to promise anything of value, no further tests were made to settle these points.

Preparation 17, formed by treating oil of chenopodium with FeSO_4 , was tested as follows:

Dog 241, a terrier weighing 13 kilos, was given this preparation at the rate of 0.1 mil per kilo, for a dose of 1.3 mils, followed immediately by 30 mils of castor oil. Two days later the dog passed 2 ascarids. The animal was killed on the sixth day after treatment and on postmortem was found to have 1 ascarid and 1 Dipylidium. The treatment was therefore 67 per cent effective against the test worms, ascarids, and 0 per cent effective against Dipylidium. The stomach showed petechiæ in the mucosa of the cardia and of the greater curvature; the duodenum was congested and the lower jejunum showed numerous petechiæ and ecchymoses. In view of the apparently injurious effect of the product on the digestive tract and its failure to show the 100 per cent efficacy that may be obtained in the same dosage from oil of chenopodium, the product was not tested further.

The most interesting and suggestive experiments are those dealing with distillation products. In the following notes, products distilling up to 125° C. at 30 mm. of mercury, or approximately equivalent products at other temperatures and pressures, are called light preparations. Those containing products distilling

above 125° C. at 30 mm. of mercury, or equivalent products, are called heavy preparations: such preparations in some cases contain lighter products in addition to the heavier.

The experiments in administering the heavy preparations were:

Dog 193, a mongrel weighing 9 kilos, was given preparation 6, distilling at 125° to 140° C. at 30 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of 0.9 mil, followed immediately by 30 mils of castor oil. The dog passed no worms and was found dead the morning of the fourth day. The animal had 1 ascarid in the stomach and 1 in the small intestine, so the treatment was 0 per cent effective. There were a few small inflamed areas in the gastric mucosa, a few petechiæ in the upper jejunum, and the large intestine and cecum were inflamed. The animal had distemper, a fact which must be kept in mind in connection with the lesions and death in this case.

Dog 196, a mongrel spaniel weighing 9.5 kilos, was given this same preparation, No. 6, at the rate of 0.1 mil per kilo, a dose of 0.95 mil, followed immediately by 30 mils of castor oil. The second day after treatment the dog passed 3 ascarids, and the third day passed 2 ascarids. The fifth day after treatment the dog vomited 20 ascarids, but the removal of these worms cannot be credited to the anthelmintic efficacy of the treatment: the usual explanation for vomiting worms would be that the stomach had been invaded by active, live worms, giving rise to irritation and emesis, and this is the logical explanation here. The dog was killed on this fifth day and found to have 7 ascarids in the small intestine, together with 2 hookworms and 11 *Dipylidium*. The treatment was therefore 14 per cent effective against ascarids and 0 per cent effective against hookworms and *Dipylidium*. The mucosa of the duodenum and upper jejunum was mildly inflamed and there were inflamed areas in the cecum.

Dog 192, a collie weighing 10.5 kilos, was given preparation 7, the residue left after distilling at 140° C. at 30 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of 1.05 mils, followed immediately by 30 mils of castor oil. The dog passed 1 ascarid the second or third day after treatment and was killed on the sixth day. Two ascarids, 4 hookworms, 6 whipworms and 1 *Dipylidium* were found postmortem. The treatment was therefore 33 per cent effective against ascarids, and 0 per cent effective against hookworms, whipworms and *Dipylidium*. The stomach showed healing petechiæ and the small intestine was moderately inflamed. There were petechiæ associated with the hookworms in the small intestine and a small inflamed area at the tip of the cecum where the whipworms were attached.

Dog 198, a mongrel terrier weighing 10 kilos, was given preparation 8, the residue left after evaporating oil of chenopodium on a steam bath, at the rate of 0.038 gram per kilo, a dose of 0.38 gram, followed imme-

diately by 30 mils of castor oil. The dog passed 1 ascarid and 1 whipworm on the third or fourth day. The animal was killed on the fourth day and found to have 26 ascarids, 16 hookworms and 1 whipworm. The treatment was therefore 4 per cent effective against ascarids, 0 per cent effective against hookworms, and 50 per cent effective against whipworms. However, this is only a little over a third of the usual dose rate used for oil of chenopodium and its preparations in these experiments. The gastric mucosa had a small area of fine petechiæ and the duodenum was mildly inflamed.

Dog 207, a bull mongrel weighing 10.5 kilos, was given preparation 11, distilling at 110° to 140°C . at 30 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of 1.05 mils, followed immediately by 30 mils of castor oil. The second day after treatment the dog passed 1 ascarid and the fourth or fifth day passed another. The dog was killed the fifth day and was found to have 1 hookworm and 7 *Dipylidium*. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against hookworm and *Dipylidium*. The ascaricidal action was slow, although this oil, rated here as a heavy preparation because containing products distilling over 125° , has also a content distilling from 110° to 125° . The stomach showed hemorrhagic areas in the pyloric region.

Dog 213, a hound weighing 17 kilos, was given preparation 12, the residue not distilling over at 145° at 30 mm. of mercury, at a rate of 0.1 mil per kilo, a dose of 1.7 mils, followed immediately by 30 mils of castor oil. The dog passed no worms and was killed on the fourth day. The animal was found to have 44 ascarids, 59 hookworms, 1 whipworm and 1 *Dipylidium*. The treatment was therefore 0 per cent effective against ascarids, hookworms, whipworms and *Dipylidium*. The stomach showed numerous petechial hemorrhages in the cardia and a congestive condition of the duodenum and jejunum.

Dog 231, a mongrel pup weighing 11.5 kilos, was given this same preparation, No. 12, at the rate of 0.1 mil per kilo, a dose of 1.15 mils, followed immediately by 30 mils of castor oil. The next day the dog had passed or vomited 3 ascarids, and the next day had passed 2 more. The animal was killed on the fourth day and found to have 16 ascarids in the small intestine, 1 in the stomach, and 1 in the large intestine, and 1 whipworm in the cecum. Crediting the ascarid in the large intestine to the treatment, the treatment was 26 per cent effective against ascarids and 0 per cent effective against whipworms. The stomach showed an area about 1.5 cm. in diameter of inflammation and petechiæ in the cardia.

Dog 235, a collie weighing 12.5 kilos, was given this same preparation, No. 12, at the rate of 0.1 mil per kilo, a dose of 1.25 mils, followed immediately by 30 mils of castor oil. The dog passed no worms and was killed on the sixth day after treatment. On postmortem examination the animal had 69 whipworms and 2 *Dipylidium*, so the treatment was 0 per cent effective against these worms. The entire digestive tract was normal at this time.

Dog 212, a mongrel bulldog weighing 15 kilos, was given preparation 14, the fraction which did not distill over at 125° C. at 30 mm. of mercury, at a rate of 0.1 mil per kilo, a dose of 1.5 mls, followed immediately by 30 mls of castor oil. The dog passed no worms and was killed on the fourth day. The animal was found to have 2 whipworms postmortem, so the treatment was 0 per cent effective against whipworms, which permits of no conclusions as to anthelmintic efficacy, since the test worm for this drug, the ascarid, was not present. The entire digestive tract was normal.

Dog 215, a mongrel collie pup weighing 7.5 kilos, was given this same preparation, No. 14, at the rate of 0.6 mil per kilo, a little more than the minimum lethal dose of 0.5 mil per kilo published by Zeigler,² a dose of 4.5 mls, preceded immediately by 30 mls of castor oil. The third day after this very large dose, the dog passed 2 ascarids. The animal was killed on the sixth day after treatment and found to have 46 *Dipylidium*. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against *Dipylidium*. The stomach showed several discrete hemorrhagic areas, 2 to 3 mm. in diameter, in the cardia; the duodenum and upper jejunum were moderately inflamed and there were very numerous petechiae in the lower jejunum and upper ileum. The protective action of 30 mls of castor oil in saving the life of a dog after the administration of more than the minimum lethal dose of a chenopodium product, is worthy of note.

Dog 221, a spaniel weighing 10 kilos, was given this same preparation, No. 14, at the same rate of 0.6 mil per kilo as in the case of the preceding dog but was not given any castor oil. The dog was profusely salivated and vomited about ten minutes after dosing. The animal was very uneasy and excited, and tried to force its way out of the cage. Later, it staggered about and would get up on its bench, lie there with its head hanging down, and then fall off. It seemed much nauseated and dizzy, and had to support itself against the walls of the cage. In an hour or so, the animal was lying down in its cage with its nose shoved in a corner and the legs moving slowly back and forth. Feces were passed about a half hour after dosing. The next morning the dog was found dead. Two ascarids and 1 hookworm were found in the small intestine and 1 hookworm in the fecal matter in the large intestine, but the animal died too soon to permit of conclusions regarding efficacy. The stomach was generally inflamed, the duodenum, upper jejunum and ileum were inflamed and in places hemorrhagic, and most of the Peyer's patches were inflamed. The speedy death of this dog bears out the protective function of the castor oil in the case of the preceding dog.

Dog 224, a mongrel weighing 10 kilos, was given this same preparation No. 14, at the rate of 0.1 mil per kilo, a dose of 1 mil, followed immediately by 30 mls of castor oil. The next day the dog passed 6 ascarids. The animal was killed on the fifth day and found free from

worms. The treatment was therefore 100 per cent effective. The stomach showed hemorrhages in the pyloric region.

Dog 226, a spaniel weighing 10 kilos, was given this same preparation, No. 14, at the rate of 0.05 mil per kilo, followed immediately by 30 mils of castor oil. The next day the dog passed 7 ascarids. The animal was killed the fourth day after treatment and was found to have 4 *Dipylidium*. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against *Dipylidium*. There were hemorrhages in the stomach.

Dog 227, a mongrel weighing 11.5 kilos, was given this same preparation, No. 14, at the rate of 0.074 mil per kilo, a dose of 0.85 mil, followed immediately by 30 mils of castor oil. No worms were passed. The dog was killed on the fourth day and found to have 3 whipworms and 5 *Dipylidium sexcoronatum*. The treatment was therefore 0 per cent effective against whipworms and *Dipylidium*. The large intestine showed ecchymotic and petechial hemorrhages.

Dog 240, a dog weighing 10.5 kilos, was given preparation 16, the residue not distilling over at 130° C. at 25 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of 1.05 mils, followed immediately by 30 mils of castor oil. The next day the dog passed 3 ascarids. The animal was killed the fourth day and was found to have 2 hookworms, 3 whipworms and 12 *Dipylidium*. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against hookworms, whipworms and *Dipylidium*. The stomach showed numerous petechiæ in the cardia.

Dog 244, a bulldog weighing 9 kilos, was given preparation 19, the residue not distilling over at 100° C. at 2 to 5 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of 0.9 mil, followed immediately by 30 mils of castor oil. The day after treatment the dog passed 7 ascarids, the second day 1 ascarid and the third day 1 ascarid. The animal was found dead on the fifth day, postmortem showing a purulent bronchiolitis with early congestive stage pneumonia, and pronounced acute nephritis. There was 1 whipworm in the cecum, the treatment being 100 per cent effective against ascarids and 0 per cent effective against whipworms. The stomach was somewhat inflamed in the pyloric region, the lower duodenum and part of the ileum were inflamed, there were small patches of submucous hemorrhage in the jejunum, and there were local inflamed areas in the large intestine and cecum.

Dog 246, a mongrel hound weighing 11 kilos, was given preparation 18, distilling below 100° C. at 2 to 5 mm. of mercury, constituting about 90 per cent of oil of chenopodium and therefore including part which would distill above 125° C. at 30 mm. of mercury, since the light fraction, distilling below 125° C. at 30 mm., is only 74 to 75 per cent of the oil. The dose rate was 1 mil per kilo, a dose of 11 mils, or double the minimum lethal dose, dissolved in 50 mils of liquid petrolatum. The dog resented the mixture and some of it went to the lungs. Immediately after dosing, the animal collapsed, and lay in a state of coma, trembling and with the

legs kicking feebly for some time. The next morning the animal was found dead. The lungs were highly congested, the kidneys inflamed, and the entire digestive tract hemorrhagic. There were 154 ascarids and 8 *Tænia*, but the period before death was too short to permit of conclusions regarding efficacy.

Dog 250, a mongrel terrier weighing 11.5 kilos, was given this same preparation, No. 18, at the same rate of 1 mil per kilo, a dose of 11.5 mils, double the minimum lethal dose, in 15 mils of castor oil, preceded immediately by 15 mils of castor oil and followed immediately by 30 mils of castor oil. The animal did not appear to be distressed after treatment. There were no feces the first day after treatment. The dog passed 7 ascarids the second day after treatment, following the administration of another 30 mils of castor oil. The day following the treatment, the animal was standing up in his cage, but trembling. In the afternoon it was lying down, apparently much depressed, but jumped up eagerly when meat was offered it and fed ravenously. The trembling persisted much of the time, though the animal was up and about. It was killed on the sixth day after treatment. On postmortem examination it was found free from worms, so the treatment was 100 per cent effective. The stomach was highly inflamed, with petechiae in the cardia; the duodenum and upper jejunum were highly inflamed, the lower jejunum moderately so, and the ileum slightly inflamed; the large intestine and cecum were normal. The constipating effect of chenopodium can be judged from the failure to pass feces during the first twenty-four hours, even after a dose of 60 mils of castor oil, while the protective action of the oil is seen in the survival of this dog after the administration of double the minimum lethal dose.

In passing, it is of interest to compare similar tests with double the minimum lethal dose of oil of chenopodium accompanied by 50 mils of liquid petrolatum in one case and by 60 mils of castor oil in the other, the only difference in these experiments from those just given being the use of oil of chenopodium instead of Preparation No. 18. The tests were as follows:

Dog 247, a mongrel bulldog weighing 10 kilos, was given oil of chenopodium at the rate of 1 mil per kilo, a dose of 10 mils, thoroughly shaken up with 50 mils of liquid petrolatum. Part of the dose went to the lungs, as in the case of dog 246. This point is of interest, for the reason that we treat a large number of dogs, and accidents of this sort are rare. Yet in one day we tested oil of chenopodium and preparation 18, giving each to 2 dogs in doses of 1 mil per kilo, each drug being accompanied by castor oil in one case and by liquid petrolatum in one case, and in both cases where it was given in liquid petrolatum, part of the dose went to the lungs. Of the 60 mils of castor oil given, 15 mils were used as a vehicle for the drugs, so the concentration of drug was higher than where the drug was given in 50 mils of liquid petrolatum. Dog 247

was staggering within fifteen minutes after being dosed, vomited about twenty minutes after being dosed, and defecated about twenty-five minutes after being dosed. The animal was evidently very sick. The next day no feces had been passed other than those passed soon after treatment. The dog was lying curled up in its cage, with its eyes closed, very much depressed and trembling. The following day the dog was comatose and died less than forty-eight hours after treatment. Fifteen minutes before death, the dog's temperature was 94.7°. The heart beat slowed to a beat every few seconds and finally stopped. All the left lobes of the lungs were in a state of red hepatization. The kidneys were highly inflamed. The stomach showed hemorrhages in places and necrosis along the top of some of the folds of mucosa; there were eroded areas in patches in the jejunum and hemorrhages in or under the glands of the Peyer's patches; the large intestine and cecum were inflamed and hemorrhagic. Feces passed during the second twenty-four hours contained 6 ascarids, and the dog was free from worms postmortem, so the treatment was 100 per cent effective.

Dog 249, a hound weighing 15 kilos, was given oil of chenopodium at the rate of 1 mil per kilo in an equal amount of castor oil, preceded by 15 mls of castor oil and followed by 30 mls of castor oil. The dog was much salivated by the treatment, but appeared to be otherwise in good condition. The next day the dog was drowsy and lay curled up in its cage. It was indifferent to food. The following day the animal seemed sick and was still lying down. It had passed no feces since it was treated, so it was given 30 mls of castor oil. On the fourth day after treatment the dog was still listless, but ate heartily. The animal was killed on the sixth day and found free from worms. No worms had been passed, so there were no conclusions as to efficacy. The entire digestive tract was normal and the other viscera showed little of interest beyond an inflammation of the medullary portion of the kidney. The experiment is a striking illustration of the protective action of castor oil against injurious effects from chenopodium.

It will be noted that in both cases where liquid petrolatum was used as a vehicle for oil of chenopodium or preparation 18 in doses of 1 mil per kilo, part of the treatment went to the lungs, and the dogs died within twenty-four or forty-eight hours after treatment and showed hemorrhages throughout the entire digestive tract; whereas, when castor oil was used as the vehicle for these drugs in the same dose, there was no difficulty in keeping the drugs out of the lungs, and when the dogs were killed on the sixth day after treatment, one showed an entirely normal digestive tract and the other showed petechiæ only in the stomach, high grade inflammation only in the stomach and upper intestine, grad-

ing to a slight inflammation in the lower intestine, and with the large intestine and cecum normal.

A summary of the ascaricidal values and the postmortem findings of the digestive tract for these heavy preparations is as follows:

In the standard dose at the rate of 0.1 mil per kilo, the ascaricidal efficacy is 0, 0, 14, 26, 33, 100, 100, 100 and 100 per cent, with no conclusions in 2 cases. In less than the standard dose: At the rate of 0.074 mil per kilo, there were no conclusions; in the 1 case in which this dose was used at the rate of 0.05 mil per kilo, the ascaricidal efficacy was 100 per cent; at the rate of 0.038 mil per kilo, the ascaricidal efficacy was 4 per cent. In more than the standard dose: At the rate of 0.6 mil per kilo, the ascaricidal efficacy was 100 per cent in 1 case, with no conclusions in 1 case; at the rate of 1.0 mil per kilo, or double the minimum lethal dose, the ascaricidal efficacy was 100 per cent in 1 case, with no conclusions in 1 case. In view of the fact that oil of chenopodium at the rate of 0.1 mil per kilo, given with 30 mls of castor oil, as most of these doses were given, had an ascaricidal efficacy of 100 per cent in nearly all cases, it must be concluded that there is a falling off in efficacy when the heavier constituents at the same rate of dosage, 0.1 mil per kilo, show values of 0, 0, 14, 26 and 33 in 5 out of 9 cases when conclusions may be drawn.

The lesions associated with these treatments are as follows: In the standard dose at the rate of 0.1 mil per kilo, we find gastritis and enteric hemorrhage in 2 dogs, enteritis and gastric hemorrhage in 1 dog, gastric hemorrhage in 5 dogs, enteritis in 1 dog, and 2 dogs normal. In doses at less than the standard rate, we find associated with doses of 0.074, 0.05 and 0.038 gram per kilo, enteric hemorrhage in the first case, gastric hemorrhage in the second case, and gastric hemorrhage and enteritis in the third case. In the excessive doses: at 0.6 mil per kilo, or more than the minimum lethal dose, we find hemorrhagic gastroenteritis once and gastritis and enteric hemorrhage once; at 1.0 mil per kilo, or double the minimum lethal dose, we find hemorrhagic gastroenteritis once and gastric hemorrhage and enteritis once. In a total of 18 dogs treated with these heavy preparations, only 2 showed a normal digestive tract, while 15 showed hemorrhagic conditions and 1 showed inflammation.

To ascertain whether the injurious effects noted are definitely to be referred to the action of the heavier constituents of oil of chenopodium, as would be inferred from the infrequency of these results in our experiments with the entire oil of chenopodium, the following tests of the lighter constituents may be compared:

Dog 191, a mongrel weighing 8.5 kilos, was given preparation 5, the oil distilling over at 110° to 125°C. at 30 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of 0.85 mil, followed immediately by 30 mls of castor oil. The next day the dog passed 14 ascarids. The dog was killed on the fourth day and found free from worms. The treatment was therefore 100 per cent effective. The digestive tract was normal throughout.

Dog 197, a mongrel weighing 17 kilos, was given the same preparation, No. 5, at the rate of 0.04 mil per kilo, a dose of 0.68 mil, to correspond to the fact that this distillate represented 40 per cent of the original oil and might be given in 40 per cent of the standard dose. It was followed immediately by 30 mls of castor oil. The next day the dog passed 138 ascarids, the second day 8 ascarids, and the third day 2 ascarids, a total of 148 ascarids. The dog was killed on the fifth day and found to have 2 ascarids. The treatment was therefore 99 per cent effective against ascarids. The first inch or two inches of the duodenum were mildly inflamed.

Dog 208, a mongrel weighing 9.5 kilos, was given preparation 9, the part distilling over below 100°C. at 30 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of 0.95 mil, followed immediately by 30 mls of castor oil. The next day the dog passed 7 ascarids. The animal was killed the fifth day and found to have 8 hookworms and 2 Dipylidium. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against hookworms and Dipylidium. The digestive tract was entirely normal except for the distinctive hookworm petechiæ.

Dog 205, a spaniel mongrel pup weighing 7.75 kilos, was given preparation 10, the oil distilling over at 110°C. at 30 mm. of mercury, at the rate of 0.1 mil per kilo, a dose of approximately 0.775 mil, followed immediately by 30 mls of castor oil. The next day the dog passed 7 ascarids. The animal was killed the fifth day and found to have 1 whipworm and 33 Dipylidium. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against whipworms and Dipylidium. There was a mild inflammation in the duodenum.

Dog 210, a mongrel foxhound weighing 12 kilos, was given preparation 13, the oil distilling up to 125°C. at 30 mm. of mercury, at the rate of 0.6 per kilo, or more than the minimum lethal dose, preceded by 30 mls of castor oil. The dog was very uneasy after treatment, moving about restlessly and trying to get out of the cage. There was a profuse salivation and the animal vomited and defecated about a half hour after treatment. The next morning the dog was very much depressed and lay curled up on his bench. Toward noon the animal was up and walking

about, but the hind quarters trembled and the animal seemed dizzy and stepped high in walking. The next day the dog seemed to be all right again. No worms were passed. The animal was killed on the sixth day and found to have 4 *Tænia pisiformis* and 2 *Dipylidium*, the treatment being 0 per cent effective against these worms. The stomach was catarrhal and the lower jejunum and upper ileum inflamed. The protective effect of the castor oil is worth noting; compare this with the following experiment.

Dog 214, a mongrel weighing 15.5 kilos, was given this same preparation, No. 13, at the same rate, 0.6 mil per kilo, a dose of 9.3 mils, which is more than the minimum lethal dose, without castor oil. There were no feces the next day, but the following day the dog passed 4 ascarids and 8 whipworms. The dog was very uneasy immediately after treatment. It moved about restlessly and tried to get out of its cage. The eyes were very luminous. The dog was very much salivated and vomited considerably in about a half hour. In about an hour the excitation gave way to depression and the animal floundered and wobbled about in its cage. The next day the animal was lying down in its cage, very weak. There was a marked trembling of the limbs and body, especially pronounced in the hind quarters. Later in the day the dog was standing, but was very unsteady, and would fall off its bench. The following day the dog was lying on its side with the legs moving back and forth; it died early in the afternoon. On postmortem it was found to have 112 *Dipylidium*, so the treatment was 100 per cent effective against ascarids and whipworms and, as usual, 0 per cent effective against *Dipylidium*. There was an intense hemorrhagic gastritis, with areas of yellowish-brown necrosis, and a general hemorrhagic enteritis.

Dog 218, a mongrel hound weighing 9.5 kilos, was given the same preparation, No. 13, at the rate of 0.1 mil per kilo, a dose of 0.95 mil, preceded by 30 mils of castor oil. The next day the dog passed 3 ascarids and 1 *Dipylidium*. The animal was killed the fourth day and on postmortem was found free from worms. The treatment was therefore 100 per cent effective against ascarids and, contrary to the usual findings, against *Dipylidium*. The entire digestive tract was normal.

Dog 220, a mongrel pup weighing 9 kilos, was given the same preparation, No. 13, at the rate of 0.74 mil per kilo, a dose of approximately 0.666 mil, followed immediately by 30 mils of castor oil. The dog passed no worms and was killed on the fourth day. On postmortem examination the animal had 6 whipworms and 1 *Dipylidium*. The treatment was therefore 0 per cent effective against whipworms and *Dipylidium*. The digestive tract was normal.

Dog 222, a mongrel weighing 10.5 kilos, was given the same preparation, No. 13, at the rate of 0.05 mil per kilo, a dose of approximately 0.525 mil, followed immediately by 30 mils of castor oil. The next day the dog passed 2 ascarids. No feces were passed after this time and the dog was killed on the fourth day after treatment. Five dead ascarids were found in the fecal matter of the large intestine and 1 dead and

partly digested ascarid was found in the small intestine, in addition to 6 live ones and 1 in the stomach. Crediting the ones in the large intestine and the dead one in the small intestine to the treatment—the rule which is followed for all the anthelmintic experiments the treatment was 53 per cent effective against ascarids. The entire digestive tract was normal, though there appears to have been an intestinal stasis resulting in the retention of all feces for 3 days.

Dog 223, a mongrel weighing 12 kilos, was given this same preparation, No. 13, at the rate of 0.1 mil per kilo, a dose of 1.2 mils, followed immediately by 30 mils of castor oil. The dog passed no worms and was killed on the fourth day. The animal had 68 hookworms in the small intestine and 1 in the large intestine. Crediting the one in the large intestine to the treatment, the treatment was only 2 per cent effective against hookworms. The digestive tract was normal except for the hookworm petechiæ in the small intestine.

Dog 225, a collie weighing 9.5 kilos, was given this same preparation, No. 13, at the rate of 0.074 mil per kilo, a dose of 0.7 mil, followed immediately by 30 mils of castor oil. The next day the dog passed 4 ascarids. The animal was killed on the fifth day and found to have 4 whipworms. The treatment was therefore 100 per cent effective against ascarids and 0 per cent effective against whipworms. The entire digestive tract was normal.

Product 15, a light preparation distilling below 130° C. at 25 mm. of mercury, was not tested, as it was substantially equivalent to other light preparations tested.

A summary of the ascaricidal values and the postmortem findings for these light preparations is as follows:

In the standard dose at the rate of 0.1 mil per kilo, the ascaricidal efficacy is 100, 100, 100 and 100 per cent, with no conclusions in 1 case. In less than the standard dose: At the rate of 0.074 mil per kilo, the ascaricidal efficacy was 100 per cent in 1 case, with no conclusions in 1 case; at the rate of 0.05 mil per kilo, the ascaricidal efficacy was 53 per cent; at the rate of 0.04 mil per kilo, the ascaricidal efficacy was 99 per cent. In more than the standard dose: At the rate of 0.6 mil per kilo, the ascaricidal efficacy was 100 per cent in 1 case, with no conclusions in 1 case. In brief, leaving out of consideration the 3 cases in which no conclusions can be drawn, the treatments, in standard doses, overdoses and underdoses, was 100 per cent effective in 6 cases, 99 per cent effective in 1 case and drops as low as 53 per cent in 1 case where half the standard dose was given. These figures might be strengthened by a larger series of experiments using

these light products in doses of 0.1 mil per kilo, but there seems to be no reason for doubting the efficacy of the product and the likelihood that such treatments would in almost all cases attain an efficacy of 100 per cent.

The lesions associated with these treatments are as follows: In the standard dose at the rate of 0.1 mil per kilo, we find the digestive tract normal in 4 cases and a mild inflammation of the duodenum in 1 case. In doses at less than the standard rate, we find with doses of 0.074, 0.05 and 0.04 mil per kilo that the digestive tract was normal in 3 cases and showed a mild inflammation of the duodenum in 1 case. In the excessive doses, we find that at the rate of 0.6 mil per kilo, with castor oil, or more than the minimum lethal dose, the jejunum and ileum were inflamed in 1 case, and at the same rate, with liquid petrolatum, there was a hemorrhagic gastroenteritis in 1 case. In a total of 11 dogs treated with these light preparations, the digestive tract was normal in 7 cases, there was a mild inflammation of the duodenum in 2 cases, and an inflammation of the jejunum and ileum in 1 case. There was a general hemorrhagic gastroenteritis in 1 case where a dose of 0.6 mil per kilo was given with liquid petrolatum. Or, briefly, of 11 dogs, 7 had normal digestive tracts, 1 had hemorrhagic conditions associated with a dose somewhat exceeding the minimum lethal dose, and 3 showed inflammation (2 of these very mild, limited conditions). In anything less than a lethal dose, the digestive tract was normal or the injury trifling.

The experiments detailed in this paper indicate rather definitely that the heavier constituents of oil of chenopodium have a diminished ascaricidal efficacy and probably a diminished anthelmintic efficacy in general, coupled with an irritant character tending to the production of inflammation and hemorrhage of the gastrointestinal mucosa, while the lighter constituents show, if no increase of anthelmintic efficacy (which is problematical), at least no diminution of the high efficacy of oil of chenopodium, coupled with a comparative freedom from irritant qualities.

A compact presentation of ascaricidal values and of lesions is given in the following table, data being tabulated on increasing dose rate. The abbreviations have the following significance: g.p.k. = grams per kilo; m.p.k. = mils per kilo; Hem. = Hemorrhage; Inflam. = Inflammation.

HEAVY PREPARATIONS.				LIGHT PREPARATIONS.			
Dog No.	Dose rate	Efficacy	Lesions	Dog No.	Dose rate	Efficacy	Lesions
		<i>per cent</i>				<i>per cent</i>	
198	0.038 g.p.k.	4	Hem.	197	0.040 m.p.k.	99	Inflam.
226	0.050 m.p.k.	100	Hem.	22	0.050 m.p.k.	53	None
227	0.074 m.p.k.	No data	Hem.	225	0.074 m.p.k.	100	None
213	0.100 m.p.k.	0	Hem.	220	0.074 m.p.k.	No data	None
193	0.100 m.p.k.	0	Hem.	191	0.100 m.p.k.	100	None
196	0.100 m.p.k.	14	Inflam.	208	0.100 m.p.k.	100	None
231	0.100 m.p.k.	26	Hem.	205	0.100 m.p.k.	100	Inflam.
192	0.100 m.p.k.	33	Hem.	218	0.100 m.p.k.	100	None
207	0.100 m.p.k.	100	Hem.	223	0.100 m.p.k.	No data	None
224	0.100 m.p.k.	100	Hem.				
240	0.100 m.p.k.	100	Hem.				
244	0.100 m.p.k.	100	Hem.				
212	0.100 m.p.k.	No data	None				
235	0.100 m.p.k.	No data	None				
215	0.600 m.p.k.	100	Hem.	210	0.600 m.p.k.	No data	Inflam.
221	0.600 m.p.k.	No data	Hem.	214	0.600 m.p.k.	100	Hem.
246	1.000 m.p.k.	No data	Hem.				
250	1.000 m.p.k.	100	Hem.				

In the column of lesions for light preparations, the inflammations present in dogs 197 and 205 were so mild and so limited that the bare statement that inflammation was present arouses a false connotation; the digestive tracts in these two dogs were practically normal. The actual production of lesions of any consequence in this series occurs when something more than the minimum lethal dose is given. In the column of lesions for heavy preparations, hemorrhagic conditions run through all the doses of 0.1 mil per kilo or less, with the exception of 3 cases where the digestive tracts were normal, or, in 1 case, only mildly inflamed.

In the column of efficacies for light preparations, in 8 cases where efficacy was obtained, it was 99 to 100 per cent in 7 cases and 53 per cent in 1 case. In the corresponding column for heavy preparations, in 13 cases where efficacy was obtained, it was 100 per cent in 7 cases, 0 to 33 per cent in the other 6.

If the heavier constituents of oil of chenopodium are principally responsible for gastrointestinal irritation and are of lessened anthelmintic value, we would expect to find that entire oil of chenopodium gives results intermediate between those obtained with the light preparations and those obtained with the heavier preparations, and in a general way this is the case. In a series of cases where oil of chenopodium was administered under substantially the same conditions as those in which the light and heavy products were administered, the results may be summarized as follows:

Thirty-three dogs were given oil of chenopodium in single or repeated doses, commonly at a rate approximating 0.1 mil per kilo, usually accompanied by castor oil, but in some instances accompanied by elaterin or elaterium. The ascaricidal efficacy was 100 per cent in the 13 dogs that had ascarids; there were no conclusions in the case of the other 20 dogs, as no ascarids were present. The digestive tract was normal in 11 dogs, there was inflammation in 12 dogs, and hemorrhage in 10 dogs. Contrasting the uniform ascaricidal efficacy of 100 per cent in these cases with the ascaricidal efficacy of the heavy and light preparations, it will be seen to exceed not only that of the heavy preparations, but also that of the light preparations, which fell to 99 and 53 per cent in 2 cases. The relatively poor showing of the heavy preparations rather precludes the idea that the entire oil is therefore more effective than its light constituents, and inclines me to the explanation that local conditions in the digestive tract, such as the presence of food or stasis, furnish a better reason for the failure of dependable anthelmintics at times than any lack of efficacy. Other experiments, where entire oil of chenopodium has fallen short of 100 per cent efficacy, which cannot be compared in this list of 33 owing to the presence of dissimilar factors, also support this view. As regards the lesions present, it will be seen that the conditions are truly intermediate between those found with the light and those with the heavy preparations. Hemorrhage occurs in less than a third, instead of in nearly all cases, as with the heavy preparations, while only a third are normal, instead of nearly all normal, as in the case of the light preparations.

CONCLUSIONS.

The experiments here noted appear to warrant the following conclusions: That oil of chenopodium as ordinarily marketed is a very potent and valuable anthelmintic, but that it not infrequently acts as a gastrointestinal irritant, a fact that seems to have been commonly overlooked, disregarded or allowed to go unstated, although noted years ago by Bruning² and of late years by Salant and Nelson;³ that the gastro-intestinal irritation seems to be due to constituents making up a fourth, or less, of the volume of the oil and constituting the undistilled fraction when the lighter boiling constituents are distilled over at temperatures up to 125°

with a pressure equal to 30 mm. of mercury, or at equivalent temperatures and pressures; and that the use of the lighter fraction as an anthelmintic in preference to the entire oil, in order to protect the patient from gastrointestinal irritation, is apparently indicated. It will be of considerable interest to obtain clinical data in regard to the therapeutic value of this light fraction, for if our findings are confirmed, the use of the refined product would be distinctly indicated, in spite of the extra expense due to distillation and to discarding part of the oil, as the protection of the patient in the administration of anthelmintics constitutes as large a part of the physician's task as the securing of worm removal. Prolonged experience with potent anthelmintics inclines one to attach adequate importance to the dangers associated with these drugs.

Schimmel and Company¹² stated, without giving any reasons or proof for the statement, that ascaridol, which apparently corresponds to the heavier fraction of oil of chenopodium, is the part responsible for the therapeutic activity of the drug, a statement which has been generally accepted. Our experiments indicate that this constituent is anthelmintic and also a gastrointestinal irritant, while the lighter portion of the oil is apparently even more anthelmintic and much less irritating. It is also worthy of note, in this connection, that Salant and Nelson⁶ found ascaridol 30 per cent more toxic than oil of chenopodium, an additional reason for using the lighter fraction of the oil.

REFERENCES.

- ¹Rose: Third Ann. Rept. Rockefeller Foundation Internat. Health Bd. 1917.
- ²Hall: New Orleans Med. & Surg. J., 1918, lxx, 637.
- ³Salant and Livingston: Am. J. Physiol., 1915, xxxviii, 67.
- ⁴Salant and Livingston: Am. J. Physiol., 1916, xli, 21.
- ⁵Salant and Mitchell: Am. J. Physiol., 1915, xxxix, 37.
- ⁶Salant and Nelson: Am. J. Physiol., 1915, xxxvi, 440.
- ⁷Zeigler: Interstate Med. J., 1917, xxiv, 961.
- ⁸Brüning: Zeitsch. f. Exp. Path. u. Therap., 1906, iii, 564.
- ⁹Hall and Foster: J. Agric. Research, 1918, xii, 397.
- ¹⁰Hoelscher: Therap. Gaz., 1917, xli, 689.
- ¹¹Vanderkleed: Mulford's Vet. Bull., 1914, vi, 53.
- ¹²Schimmel and Company: Semi-Ann. Rept., 1908, Apr., 109.
- ¹³Nelson: Bu. Chem. Circ. 73, 1911.
- ¹⁴Kremers: Pharm. Rev., 1907, xxv, 155.
- ¹⁵Nelson: J. Am. Chem. Soc., 1911, xxxiii, 1404.
- ¹⁶Hall: Musser and Kelly's Practical Treatment, 1917, iv, 389.
- ¹⁷Hall: J. Am. Vet. Med. Ass., 1917, v, 342.
- ¹⁸Hall and Foster: J. Am. Med. Assn., 1917, lxxviii, 1961.

**Studies from the Research Laboratory.
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**THE STABILITY OF CANNABIS SATIVA AND ITS
EXTRACTS.**

BY HERBERT C. HAMILTON.

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A recent publication by Eckler¹ regarding the deterioration of *Cannabis indica* raises a question which can be answered positively only after a long series of experiments such as Eckler himself conducted.

Over a period of five years, samples of crude drug cannabis were kept under observation, storing it under different conditions. He found that it loses from 1 to 2 per cent of its activity monthly, depending apparently on the temperature of the storage room. Five years from now, however, the subject will probably have passed so completely out of general interest that there will be no incentive either to complete the experiments or to make the results public, while at the same time such an apparently authoritative statement going unchallenged may lead to a number of errors. It seems advisable, therefore, to publish some data bearing on this question even if it is not based on systematic experiments. In the course of nearly 20 years' experience in applying the physiological assay process to cannabis preparations, a number of unrelated facts are gradually collected which, taken as a whole, have a value not to be ignored.

Cannabis indica, or to use the botanical term to cover *Cannabis sativa* wherever grown, is scarcely deserving of the attention it has received from time to time in recent years. While a potent drug in many respects, its action is not specific in therapeutics and is not that of a deadly poison.

However, as a potent drug in which no active substance with well-defined chemical characteristics has been recognized, it requires standardization and sufficient investigation to insure that inert extracts be kept off the market.

Attention was first called to the variability of the drug by Houghton,² who found that about 50 per cent of the fresh samples of crude drug were devoid of activity.

Later, Houghton and Hamilton³ described a method by which extracts can be standardized and uniformity established in the quality of commercial extracts. It depends on the fact that dogs react to the drug in a degree proportionate to the amount of a standard product administered, and that the reaction is not only characteristic but measurable.

This method, on which all the later modifications have been based, has served as a means of eliminating most of the worthless drug from the market and of discovering evidences of deterioration. Thus it was observed that Powdered Extract Cannabis is apparently an unstable form since several samples have been found inactive. In one case⁴ some step in the process of manufacture caused immediate and almost complete loss of activity.

No other evidence of deterioration has been observed, however, except that which tends to corroborate the data published by Marshall⁵ and quoted by Eckler. The deterioration of the substance to which the name of cannabinol was given, while easily demonstrable, does not prove anything about the drug itself or its extracts, except when similar phenomena are shown to exist in each. The conclusion which Marshall drew from his observation that "There is good reason to believe that preparations of *Cannabis indica* relatively quickly deteriorate" is not based on any data submitted and is not justified by any data obtained except the two preparations already mentioned. Marshall himself did not apply it to the drug, while Eckler concludes that the crude drug alone is under suspicion, not having observed any deterioration in the fluidextract.

Observations on the crude drug other than samples of indefinite age are limited to two, which will be described below. During a long experience in assaying samples of the crude drug before or immediately after purchase, no low activity has been observed except such as is easily referable to its physical or botanical properties. For example, low activity may be expected from samples consisting of sweepings or from those consisting largely of stems or seeds, or from those having a low yield of alcohol-soluble extractive. (NOTE: By alcohol-soluble extractive is meant the part which will redissolve in 95 per cent alcohol after

twice evaporating to complete dryness on the steam bath.) Low activity of crude drug other than the exceptions noted would prove nothing in itself because of having no data as to the age of the sample.

Inquiry among holders of crude drug rarely brings to light samples much over 1 year old in stock, and the standard is usually made from drug no fresher than this.

Two samples, however, have recently been obtained from shelf bottles, one 14 years on the shelf and the other at least 21 years and probably longer, 21 years being the limit of the botanist's knowledge. The results of an assay of these two samples in comparison with standard are as follows:

14-year old drug.....	70 per cent.
21-year old drug.....	20 per cent.

The first was known to the writer to be of good quality, probably fully standard. The second lot was probably also a first-class quality of *Cannabis indica* from its appearance, but there were no recorded tests in existence to prove its activity.

Exact data on old F. E. *Cannabis indica* is limited to one sample other than an occasional retest of samples not over 2 or 3 years old, which are almost invariably as active as when fresh. The old sample referred to above was obtained from Hutton and Hilton, retail pharmacists of Washington, D. C. It was prepared by Squibb and had been on the shelf for 17 years. The assay showed a value equal to about 70 per cent or between two-thirds and three-fourths as strong as standard. Its original value may be assumed to have been 100 per cent.

Exact data on Extract *Cannabis sativa* is limited to one sample, an extract of *Cannabis Americana* first selected for a series of experiments to prove the applicability of the physiological assay to drug intended for clinical use.⁶ This sample has been in constant use in our laboratory for 9 years as a standard for the selection of dogs for assaying cannabis. It is at this time as active as an extract of the best obtainable commercial lots of *Cannabis indica*. It is rare indeed when any extract exceeds it in activity. The sample is kept in a tin can with tightly fitting lid, but is opened on an average not less often than once every week.

The bottles containing the crude drug are 1-pound flint glass, glass-stoppered bottles which have never been sealed and have

been kept on shelves exposed to the light and variations of temperature normal to a laboratory work room.

That the above results are not open to the suspicion of being obtained on the basis of a standard of low activity is demonstrated by the fact that comparison was made with a standard which has recently been proposed and prepared by Pearson.⁷ This is in line with a similar suggestion by Lyons⁸ and with that by Hamilton.⁹ It eliminates the variable standard suggested in the Ninth Revision of the U. S. P., and provides identical material for comparison in the several laboratories.

The results summarized above are based on administering doses to dogs and observing the degree of the reaction, comparing the effect in each case with that of a product of known good quality. The dogs' behavior under cannabis must have been previously observed since no two react in exactly the same way. The reaction which is described as "well-marked" or "standard" refers to the incoordination and is not absolute but only relative to that produced by the Standard on that dog.

The doses used and the observed reactions are the bases of the results described in the foregoing article and are given in detail below, the work having been carried out by my colleague, L. W. Rowe:

Sample No. 1 is F. E. *Cannabis indica* from 14-year-old drug.

Sample No. 2 is F. E. *Cannabis indica* from 21-year-old drug.

Sample No. 3 is F. E. *Cannabis indica* from Hutton and Hilton, Washington, D. C.

Sample No. 4 is S. E. *Cannabis Americana*.

Sample No. 5 is F. E. *Cannabis indica*, mixture of 4 commercial samples supplied by Pearson.

	Dose, in ml.	Results
Sample 1	0.08 ml	Slight incoordination
	0.10 ml	Distinct
	0.12 ml	Well marked
	0.14 ml	Standard
Sample 2	0.10 ml	No reaction
	0.20 ml	Very slight
	0.30 ml	Slight
	0.40 ml	Well marked
	0.50 ml	Standard
	0.60 ml	Very marked incoordination
Sample 3	0.10 ml	Distinct
	0.12 ml	Well marked
	0.15 ml	Very marked incoordination
	0.12 ml	Well marked but scarcely standard

	Dose per kilo.	Results.
Sample 4.....	0.008 mil	Well marked
	0.010 mil	Standard
	0.012 mil	Very marked incoördination
Sample 5.....	0.08 mil	Well marked
	0.10 mil	Standard
	0.12 mil	Very marked incoördination
Sample 5.....	0.025 mil	No reaction
	0.03 mil*	Slight incoördination
	0.04 mil	Distinct incoördination

*This is the result using the U. S. P. dose and observing the conditions rigidly.

The above data leads to the conclusion that the rate of deterioration of *Cannabis sativa* and its official extracts is much slower than would be assumed from Eckler's work and that for practical purposes it may be ignored.

REFERENCES

- ¹Eckler, *J. A. Ph. A.*, Vol. 6, p. 812.
- ²Houghton, *J. A. M. A.*, Vol. 28, p. 634.
- ³Houghton and Hamilton, *A. J. of P.*, Jan., 1908.
- ⁴Hamilton, *J. A. Ph. A.*, Vol. 4, p. 448.
- ⁵Marshall, *Pharm. Jour.*, Vol. 28, p. 418.
- ⁶Hamilton, Lescotier and Perkins, *J. A. Ph. A.*, Jan., 1913
- ⁷Pearson, *J. A. Ph. A.*, Vol. 6, 1917, p. 876.
- ⁸Lyons, *J. A. Ph. A.*, Vol. 6, 1917, p. 877.
- ⁹Hamilton, *Am. Journal of Pharmacy*, Feb., 1917.

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SOME NOTES ON CHENOPODIUM.

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Chenopodium is also known as American wormseed, Mexican tea, Spanish tea, Jesuit tea, Jerusalem tea, and Jerusalem oak. These terms are applied to the plant *Chenopodium anthelminticum* Linnæus, also known as *Chenopodium ambrosioides anthelminticum* A. Gray, a member of the Chenopodiaceæ or Goosefoot family of the order Chenopodiales. In the same genus is the ordinary lamb's-quarters, or pigweed, used as a food plant in this country and formerly so used by the Indians. Other species of this genus have been used in medicine as anthelmintics, antispasmodics, expectorants, emetics, emmenagogues, and in treatments for chorea. Chenopodium is said to have been naturalized in this country from tropical America, and to occur in waste places from New England to Florida and California. It is very common in Virginia, Maryland, the District of Columbia, Arkansas, and Florida, and is cultivated for the market in parts of Maryland, especially in Carroll county. Moillet and Carreno state that as "epazote," chenopodium is known almost all over Mexico.

The plant grows to a height of 2 to 3 feet (Brüning¹ says 2 to 5 feet, quoting an early Dispensatory) and may be distinguished by the odor of the volatile oil of chenopodium which is noticed when the leaves, stem, or seeds are crushed. According to Brüning, the plants yield 1.5 to 2 per cent of this oil. Keith² states of oil of chenopodium: "It is an ethereal oil obtained from an American plant resembling the cabbage." As chenopodium bears no resemblance to cabbage and belongs to a different order of plants, this statement appears to be based on misinformation. A recent statement in the *Boston Medical and Surgical Journal* (v. 175 (23), p. 438) to the effect that the principal sources of chenopodium are in the islands of Java and Sumatra and the Levant is probably based on a confusion of American chenopodium and the Levant *santonica*.

¹Brüning, H.: *Zeits. f. Exper. Path. u. Therapie*, 1906, 3, pp. 564-587.

²Keith, R. D.: *J. Trop. Med. and Hyg.*, 1916, 11, pp. 130-131.

In anthelmintic medication, the bruised fruit has been given in doses of 20 grains in an electuary; or the expressed juice of the fresh plant used in tablespoonful doses; or the decoction made by boiling an ounce of the fresh plant in a pint of water given in 2-ounce doses; or the fluid extract given twice a day for three days in doses of from 15 minims to a drachm; or the oil of chenopodium derived from the plant by distillation has been given, according to various published reports, as follows:

With preliminary purgation with Epsom salts, and without preliminary purgation: in doses of 3 to 10 drops, 15 drops, 16 drops, 10 minims, 15 minims, 16 minims, 17 minims, 21 minims, 30 minims, and 0.22 mil to 1 mil, for adults; the dose is given once or repeated for a total of two or three doses; the interval between doses is a half-hour, an hour, two hours, four to five hours, and twenty-four hours; the oil is given in sugar, molasses, honey, milk, emulsion, capsules, or globules; it is followed by Epsom salts, Glauber's salts, castor oil, castor oil and chloroform, black draught, unspecified cathartics, or by no purgation whatever; the interval between the last dose of oil and the dose of purgative is usually two hours, sometimes four hours; the oil of chenopodium is occasionally combined with castor oil, chloroform, and oil of eucalyptus. Most writers advocate the use of three doses with a one- or two-hour interval between doses. It may be noted in passing that a minim and a drop of oil of chenopodium is not at all the same thing. A casual test with a pipette shows over two drops to the minim, a given number of minims, therefore, constituting more than double the dose of the same number of drops. The maximum dose is that given by Keith—three doses of 30 minims each at hour intervals. Heiser³ states in a discussion of Keith's paper that Keith gives 10 minims each morning for three mornings, and then follows the last dose with castor oil after a two-hour interval, but Heiser appears to have quoted Keith wrongly in this respect. The dose method attributed to Keith by Heiser has been recorded by Thornburg⁴ and by Coutant,⁵ the latter reporting a case of severe poisoning from this treatment. I regard this method of treatment, giving chenopodium every morning for three mornings with no purgation until the third day, as the worst of the methods so far proposed, for reasons already published by Hall and Foster⁶—*i. e.*, that oil of chenopodium is

³Heiser, V. G., *Annals of the Entomological Society of America*, Vol. 34, No. 1, 1941, pp. 266-277.

⁴Thornburg, C. C., *Annals of the Entomological Society of America*, Vol. 34, No. 1, 1941, pp. 266-277.

⁵Coutant, A. L., *Annals of the Entomological Society of America*, Vol. 34, No. 1, 1941, pp. 266-277.

⁶Hall, M. C., and W. D. Foster, *Annals of the Entomological Society of America*, Vol. 34, No. 1, 1941, pp. 266-277.

distinctly constipating and should be given with a purgative rather than left for two 24-hour intervals before purgation. Experimental work in this laboratory indicates that the prompt administration of castor oil has very distinct protective value against the irritant and toxic properties of oil of chenopodium, saving the lives of dogs that were given doses found to be lethal when given without castor oil, and it appears to be superior to any other purgative for use with oil of chenopodium. In numerous experiments we have found the administration of the oil of chenopodium in soft or elastic capsules to be the most satisfactory mode of administration. On the other hand, Darling, Barber and Hacker⁷ found magnesium sulphate superior to castor oil in clinical experience, and did not obtain as satisfactory results from the soft capsules used by them as from hard capsules. This matter needs additional consideration, owing to differences in the composition of various makes of soft capsules and owing to the effect of the contained drug on the solubility of capsules of any given composition.

Millspaugh⁸ states that chenopodium is said to have been used by the American Indians as a vermifuge and as a treatment for dysmenorrhea. Weeds that are notoriously bitter or pungent are very commonly regarded by primitive peoples as potent plants, either as drugs or for other purposes, and it is unlikely that such a highly and distinctively odorous plant as chenopodium would go uninvestigated. And it would only take a moderate amount of investigation, in all probability, to establish the potency and the particular value of chenopodium. Primitive peoples, children and persons of low mentality and little education are especially subject to infestation with parasites, this being a result of the unsanitary surroundings and habits common among those mentioned. Where infestations with such worms as ascarids are common, as was probably the case among the Indians, the connection between the ingestion of chenopodium and the passage of these large worms would not be hard to imagine or establish. Millspaugh states that the Indians also used *spigelia* (pinkroot), another native anthelmintic, before Columbus discovered America.

Following the Indian's use of chenopodium, there has been a long "wormseed tea" period, during which period this valuable anthelmintic was largely used as a home remedy on the farms

⁷Darling, S. T., M. A. Barber, and H. P. Hacker: *Journal A. M. A.*, Feb. 23, 1918, pp. 499-507.

⁸Millspaugh, Chas. F.: *Medicinal Plants*, v. 2, 1892.

and by the colored "mammies" of the South. At the same time our medical men very generally ignored this valuable anthelmintic and used imported, and in the main less effective, drugs. An appreciation of chenopodium, which began before the outbreak of the present war, has been intensified by the shortage of imported drugs since the war began, and at present chenopodium is being very largely used and is receiving an increasing amount of study and attention.

According to Nelson,⁹ oil of chenopodium was first investigated by Garrigues in 1854 and reported as containing a hydrocarbon boiling at 176° C. and a liquid of the formula $C_{10}H_{16}O$. The same authority states that Schimmel & Company, by fractionating at reduced pressure (the oil exploding on attempted distillation at atmospheric pressure), separated a liquid, designated as ascaridol, which constitutes the major portion of the oil and to which the peculiar chemical and medicinal properties are due. Ascaridol is a yellow oil with the formula $C_{10}H_{16}O_2$. Nelson came to the conclusion that it was an unstable dioxide.

Investigations of the action of oil of chenopodium naturally follow two independent but correlated lines of work. One line is an investigation of its actual efficacy as an anthelmintic, since that is practically the only use of the drug; the other line is an investigation of the physiological action of the drug on the host animal, since anthelmintics are practically always poisonous to some extent, and it is desirable that we know their incidental effects as well as the dose and mode of administration that will give adequate anthelmintic action with a minimum injurious effect on the host. This last line of investigation has been pursued by Salant and his collaborators in the U. S. Bureau of Chemistry for some years. Studies of the anthelmintic efficacy have been made by a number of physicians in this country and abroad with clinical findings as a basis, by Hall and Foster in the U. S. Bureau of Animal Industry, and by myself in this laboratory with animal experimentation as a basis. These experimental findings have been published in part in the preliminary note by Hall and Foster and will be given in detail in subsequent papers. The experiments indicate that in animals having a simple digestive tract, as in the case of man and the carnivora, oil of chenopodium is more effective against such worms as ascarids than any other anthelmintic now in use.

⁹Nelson, F. K. *Bull. Chem. Educ.*, 73, 491, (1911).

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CANINE COCCIDIOSIS, WITH A NOTE REGARDING OTHER PROTOZOAN PARASITES FROM THE DOG.*

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In a recent paper, Hall (1917) reported the occurrence of a coccidian in dogs in Detroit and stated that its size precluded the idea that it was *Diplospora bigemina*, so far as available literature showed. An examination of Stiles's (1892) original paper and of the measurements given by Fantham (1916) leads us to the conclusion that this coccidian at Detroit is *D. bigemina*. In the first 200 dogs examined here, this parasite was found in 15, or 7.5 per cent, a frequency that warrants some study of this organism and which suggests that it is probably much commoner in American dogs than our present lack of information would suggest. Another thing which warrants consideration of this protozoan is the fact that it is reported as one of the species occurring in man. In 1915, Wenyon reported a coccidian, *Eimeria* (*Coccidium*), from soldiers on the Gallipoli Peninsula, and the same year Woodcock reported *Diplospora*, a generic name sometimes used in place of *Diplospora*, from soldiers in the same locality. (The available literature states that *Diplospora* develops 2 spores each containing 4 sporozoites, while *Isospora* develops 2 spores each containing a variable number of sporozoites.) Castellani (1917) says that coccidiosis in man is comparatively common in the Balkans and notes a number of cases, stating, in comment, that treatment was unsatisfactory. Inasmuch as our soldiers may yet fight on any of the battle fronts surrounding the Central Powers, the diseases to which they may be exposed deserve especial consideration at this time. Moreover, the disease in the dog has received very little study.

In the case of dog No. 127, a heavily infected animal, this

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coccidian showed a marked tendency to occur in adherent groups in the feces. We use the method of examining the feces described by Hall (1917) and in spite of the use of the mechanical agitator, coccidia were commonly found in clusters of two to twelve in the feces of this animal. We regard this as perhaps due to an adhesive character of the oocyst wall or to a particularly mucilaginous quality of this dog's feces.

Stiles (1892) notes that this species occurs in dogs and cats and in the polecat, *Putorius putorius*. Railliet and Lucet (1891) have reported these as different varieties, *Coccidium bigeminum canis*, *C. bigeminum cati*, and *C. bigeminum putorii*. Weidman (1915) has reported another variety, *C. bigeminum canizelocis*, from swift foxes in the western United States. Stiles notes that the coccidia from the intestine of the dog which have been referred by various authors to *Coccidium perforans*, the form from the rabbit intestine (regarded by some workers as identical with *Eimeria stiedae*), are probably *C. bigeminum*. Recently Guillebeau (1916) has reported *Eimeria stiedae*, the coccidian commonly found in the liver of rabbits, from the liver of dogs, but in his review of this paper (Guillebeau, 1917) Railliet concludes that the organisms described are not coccidia, but blastomycetes. Railliet does not regard the hepatic organisms described by Lienaux, Clark, Marcone, or Olt as coccidia, and notes that forms described by Perroncito are trichosome eggs. He believes that



FIG. 1. *Diplospora bigemina*.—Oocyst with two spores, each containing four sporozoites and a residual body. Highly magnified.

Diplospora bigemina from the mucosa and submucosa of the intestinal villi is the only coccidian known from the dog, and that while hepatic coccidia may occur in the dog, no case of the sort has been established. In passing we note the following editorial from the *Pacific Medical Journal* of July, 1916:

"Coccidiosis. This disease, said to be more or less prevalent

in San Joaquin Valley of California, is produced by a protozoan or psorosperm. The parasite is found in the excreta of dogs, hogs, and in the intestine and liver of rabbits and in bones of the human family. Some forty cases are known. Most of them originated in the San Joaquin Valley. The mortality is 100 per cent. The most recent case occurred in Los Angeles County Hospital."

There appears to be some misunderstanding or error involved here.†

Most of the material we have examined falls within the range of measurements given by Hall (1917), the oocysts measuring 36 to 40 μ long by 28 to 32 μ wide. Fantham (1916) gives a range of 22 to 40 μ by 19 to 28 μ . These forms have 2 spores, each spore measuring 10 to 20 μ in diameter (Fantham gives a range of 10 to 18 μ), and each spore contains 4 sporozoites, each 12 μ long by 4 μ wide, and a residual body (Fig. 1). In one animal, dog No. 223, we found a smaller strain of coccidia, the oocysts measuring 20 by 18 μ in diameter, the spores 12 by 11 μ in diameter, and the sporozoites being 10 μ long and 3 μ wide. The distinction in size between the coccidia in this animal and the other coccidia was quite marked and naturally raises the question whether this should be regarded as a strain, a variety, or a species. It is possible that there are several species of *Diplospora* in the dog, characterized by considerable differences in size, or there may be one species developing various strains under certain determining conditions. This is not a matter on which we care to pass judgment, but it is a question which apparently deserves investigation. If we understand the figures given by Stiles and other writers, the oocysts from European dogs are about 10 μ in diameter, the small ones at this point are about 20 μ in diameter, while the large ones here and the forms reported by Weidman (1915) from the western swift fox, examined in the Philadelphia Zoological Garden, attain a diameter of 40 μ .

Some incidental attempts to culture our coccidian material gave the following results: Coccidia of the larger strain, in feces from dog No. 127, were kept in a 10 per cent solution of potassium bichromate, as advocated by Cole and Hadley (1910), at

†This may refer to infection with *Coccidioides*, which is at present regarded as a fungus.

room temperatures. The culture was not examined until the ninth day, at which time most of the oocysts had spores containing sporozoites. A second bichromate culture was made up of coccidia-infested feces of dog No. 210 and kept at a temperature of 23° to 29° C. During the first 24 hours the oocyst material divided to form the two sporoblasts. During the next 24 hours, the spores formed and the sporozoite development went on to the production of two to four cells to a spore. During the third day, sporozoites were present in almost all oocysts. Under such favorable conditions, then, feces containing this coccidian would probably be infective for other susceptible animals by the fourth day. A third culture from the feces of this same dog, No. 210, was made at the same time as the one just noted, but was made with tap water. At the same temperatures as the preceding culture, this culture with water showed no apparent change the first 24 hours. During the next 24 hours some cell division was observed in the oocysts. The subsequent development proceeded very slowly and the early stages of sporozoite development were not noted until the eighth day. On the fourteenth day several oocysts were found showing developed sporozoites. A fourth culture was made with a smaller strain of coccidia, the infected feces of dog No. 223 being cultured in 10 per cent potassium bichromate at temperatures of 20° to 23° C. During the first 24 hours, cell division had proceeded to the point where there were 2 well-defined sporoblasts. During the second 24 hours, development proceeded to the formation of 4 well-defined sporozoites. This material was fed to dog No. 153, which it failed to infect, but this dog seemed to have acquired immunity to coccidiosis. It appears, then, that feces of dogs infected with *Diplospora bigemina* may become infective for susceptible animals under very favorable conditions in 48 hours, probably, and under unfavorable conditions may remain uninfected for two weeks or longer. It is unlikely that conditions in nature very often approach the favorable conditions afforded by the use of potassium bichromate solution and the maintenance of the temperature noted, and consequently the development period in nature probably is a matter of weeks rather than days as a rule.

To ascertain the length of time required for the coccidia to develop from the infecting sporozoites in the dog to the appear-

ance of oocysts in the feces, the following experiments were performed:

Dog No. 216, under treatment with Fowler's solution, was given a heavy concentration of sporozoites about 1 hour after receiving Fowler's solution. The dog vomited about 15 minutes later, probably losing some of the coccidian material in this way. Fecal examinations for the following 13 days were negative. Dog No. 219, having shown no coccidia on a single fecal examination, was fed a heavy infestation of sporozoites from the feces of dog No. 127. The feces showed no coccidia the next 3 days; the fourth day was Sunday and the feces were not examined; the fifth day oocysts were found in the feces, the cycle from sporozoite to oocysts in the feces requiring at least 4, possibly 5, days. Dog No. 130, having shown no coccidia on a single fecal examination, was fed a half drachm of a bichromate culture of feces containing coccidia. No coccidia were found in the feces the next two days and no feces were passed the third day. On the fourth day, oocysts appeared in the feces, among them an oocyst containing 2 spores, which suggests that the oocyst in the 1-celled stage, as ordinarily found in the feces, was present on the third day. The post-mortem examination of this dog on the fifth day after feeding the coccidia showed numerous coccidia in various stages of development in the intestinal wall. Dog No. 173 was given a half drachm of culture containing coccidia in bichromate solution. Fecal examinations through the following 10 days were negative. The feces were not examined the eleventh day, but on the twelfth day they were examined and oocysts found. The developmental period from the ingestion of sporozoites, therefore, appears to require from 4 days, possibly as little as 3 days, to 11 or 12 days. A culture of coccidia in potassium bichromate was fed to a rat, but fecal examinations were still negative at the end of 19 days, and the examinations were abandoned with the presumption established that rats are probably not capable of infection with *D. bigemina* under ordinary circumstances.

Although the treatment of coccidiosis is generally unsatisfactory at present, it has been noted by investigators that infestations are commonly self-limited; after the host is infested, the coccidian reproduces by schizogony for a time, spreading the infestation more widely in the host, but after a time the parasitic elements

undergo the sexual transformation leading to the formation of oocysts intended for the dissemination of the infection to other host animals, the schizogonous cycle comes to an end, and the infestation terminates. We had occasion to observe this for *D. bigemina* in the case of dog No. 173. As noted above, feeding of infective material led to the infestation of this dog and the appearance of oocysts in the feces on the twelfth day. Oocysts were numerous in the feces of this dog for a little over a week. On the tenth day of oocyst occurrence in the feces there was a marked falling off of their number and very few were found the next two days. No oocysts were found thereafter, so the infestation required 12 days before oocysts appeared in the feces and lasted only another 12 days so far as the presence of oocysts showed infestation. Fecal examinations for the next 61 days showed no oocysts, in spite of attempts at reinfestation.

Crawley (1912) states in regard to the self-limitation of coccidiosis: "Apparently, however, no immunity is produced, and another attack of the disease may result from a fresh infection from without." An experiment along this line was carried out in connection with the self-limitation of infection of dog No. 173. After this dog had been infested by feeding, had shown the infestation for 12 days, and had then been apparently free from infestation for 11 days, the animal was given 2 mls of material from the same infective culture of *D. bigemina* that had previously infected it. No oocysts were found in the feces during the next 18 days. Nineteen days after this attempt to infect the dog, the animal was given 3 mls of a heavy concentration of coccidian material obtained from this animal while it was showing an abundance of coccidian material in the feces. No oocysts were found in the feces during the next 16 days. Seventeen days after this second attempt, the animal was given 4 mls of a culture of the small strain of coccidia found in dog No. 223. No oocysts were found in the feces during the next 11 days, at which time the dog was killed. This dog had shown no sign of coccidiosis after having been fed 3 cultures at intervals of 11, 28 and 47 days previous to death. The animal had been susceptible to coccidiosis when first given infective material after being found negative. In all other cases where we fed the same coccidian material to dogs, we infected the animals without difficulty, except in the case

of dog No. 216, which could not be infected with oocysts from infected feces of this dog (No. 173). In this case (No. 173) we failed to infect the dog with the same strain that had originally infected it, with the coccidia taken from the dog's own feces, and with a totally different strain, morphologically distinguishable and from a different animal. The obvious explanation that occurs to us is that this dog had developed an immunity to *D. bigemina*. Other explanations may be figured out, but they seem less plausible than this, and we submit that this is apparently a case of acquired immunity to coccidiosis. The apparent immunity to the small strain is evidence, though not conclusive, that this and the larger form are only different strains of a single species.

As regards the pathological significance of *D. bigemina*, we have but little information, but the following notes may serve some purpose: Dog No. 130 presented a clinical picture of distemper and died of pneumonia, probably due in part to distemper and partly to an accident in drenching. The small intestine showed diffuse hemorrhagic points, most pronounced in the ileum, especially the lower ileum near the valve. Scrapings of the mucosa showed the coccidia to be most abundant in the ileum, less so in the jejunum and least so in the duodenum. These findings of increasing numbers of coccidia with increasing severity of lesions may be correlated, but in the absence of sections indicating the relation of the coccidia to the hemorrhage, we do not care to hazard a definite opinion. Dog No. 173 showed numerous fine petechiae in the intestinal mucosa, and these were especially numerous in the Peyer's patches, giving these a uniformly dark appearance. No sections were made and this dog had shown no oocysts in the feces for 45 days. Dog No. 127 showed innumerable pinpoint petechiæ in the ileum, but it would be unsafe to draw conclusions based on this one dog, as the animal figured in other experiments. The intestine of dog No. 223 was macroscopically normal except for the presence of hook-worm petechiæ. Other dogs that had coccidiosis were used in experiments that complicate the post-mortem findings and leave the effect of the coccidiosis uncertain. However, we may note that Weidman (1915) found what he regards as a variety of *D. bigemina* in hemorrhagic, ulcerative enteritis in the swift fox, raising the question as to the possible etiological relationship. In view

of the fact that coccidia are destructive to epithelial tissue and that some species fairly closely related to *D. bigemina* are known to be highly pathological, it would seem reasonable to suppose that *D. bigemina* might be distinctly pathological at times, though the apparent good health and lack of post-mortem lesions in other dogs makes it certain that it often does no visible damage.

A few clinical observations may be noted in this connection. As regards the presence of febrile conditions, we find the following: Dog No. 173 showed a preliminary temperature of 101.5° before the feeding of coccidia to the animal. During the next ten days, in which no oocysts were found in the feces, the animal's temperature was as follows, no temperature being taken on the fourth day: 101.7°, 101.0°, 100.3°, 101.2°, 102.4°, 103.0°, 101.1°, 101.6°, 99.8°. This shows a slight rise in temperature on the sixth and seventh days. No temperature was taken on the eleventh day. On the twelfth day oocysts were found in the feces and were abundant for the next 7 days. This period was marked by a steady rise in temperature as follows: 100.0°, 101.0°, 101.2°, 101.4°, 101.8°, 102.0° (no temperature taken the eighteenth day after feeding), 102.4°. The following days there was a marked falling off in the number of oocysts in the feces and a simultaneous drop in temperature as follows: 101.5°, 100.8°, 101.0°. Oocysts then disappeared from the feces, the temperatures persisting within the limits of normal temperatures as follows: 100.3°, 100.0°, 101.9°, 101.0°, 100.0°, 100.8°, 100.4°. Dog No. 219 showed a preliminary temperature of 102.0°. This animal had distemper and a pronounced goitre, conditions which complicated the clinical picture of coccidiosis. After feeding the coccidia and while no oocysts were found in the feces, the animal's temperature dropped and then rose as follows: 101.0°, 101.8°, 103.4° (no temperature taken the fourth day after the feeding). The next day oocysts were found in the feces and again the temperature dropped and rose as follows: 101.0°, 102.4°, 102.8°, 102.7°, falling the next day to 101.6°. Three days later the oocysts were very scarce in the feces and the temperature fell to 100.0°. The animal was put on anthelmintic treatment at this time, introducing another complication, but no more oocysts were found in the feces and the temperature remained under 100.0°. Dog No. 130 apparently got some of the drench containing coc

cidia into its lungs. No preliminary temperature was taken. The day after the animal was given the coccidia, the temperature was 101.0°, the next day 103.2°, the next day 101.0°. During this time there were no oocysts in the feces. The following day, oocysts appeared in the feces and the temperature rose again to 102.4°, the animal dying of pneumonia the following day. Post-mortem examination showed areas of red hepatization in all the lobes of the lungs.

A consideration of the temperatures given suggests that there is a slight elevation of temperature during the period that oocysts are found in the feces, but it would be unsafe to generalize from so few cases. A consideration of the temperatures of a number of dogs not infested experimentally, but found infested on fecal examination, shows the following temperatures for periods when oocysts were present in the feces (some feces were only examined on one day for oocysts): Dog No. 66—101.3°, 101.5°, 101.4°, 101.0°, 101.5°, 102.5°, 98.8°, 95.0°, followed by death (case complicated by pneumonic condition of left median lobe and by administration of coal-tar preparation); No. 112—101.9°, 101.0°, 102.0°, 101.4°, 101.0°; No. 119—102.2°; No. 127—102.5°; No. 129—101.5°; No. 139—101.3°; No. 167—101.2°; No. 168—101.6°. Taking the figures given by Malkmus (1912) as the normal for the dog, 99.5° to 102.2°, we are impelled to the conclusion that intestinal coccidiosis in dogs, due to the presence of *Diplospora bigemina*, is an afebrile condition.

In the experiment dogs which were given some attention with reference to the presence of coccidia, diarrhea was noted as follows: Dog No. 173 developed a diarrhea on the sixth day after oocysts appeared in the feces, and had a bloody diarrhea on the last day in which oocysts appeared in the feces. Dog No. 219 developed a diarrhea on the second day after oocysts appeared in the feces; the following day there was much gas in the feces; the following day there were blood and gas in the diarrheal feces, the blood persisting in the diarrheal feces over the period in which oocysts were found and while the experiment remained uncomplicated by other factors. Dog No. 130 developed a diarrhea on the first day that oocysts appeared in the feces. Inasmuch as diarrhea is a prominent symptom of coccidiosis in man, cattle, rabbits and birds, and is a logical sequel of the coccidian injury to the intes-

tinal mucosa, we are of the opinion that the diarrheas observed may reasonably be associated with the coccidiosis as perhaps its most prominent symptom. It is a point that deserves further investigation, but it may be tentatively accepted as in accord with the probabilities. In the case of this species which undergoes much of its development in the submucosa, there appears to be no diarrhea while only the developmental stages in the submucosa are present, but the passage through the mucosa of the oocysts gives rise to injury resulting in the production of diarrhea. Light infestations may not show diarrhea; the organism is present, but no clinical picture of coccidiosis is presented.

For some reason, the protozoan parasites of dogs in this country have received very little attention and there is little known on the subject. It is possible that such parasites are as scarce as the scanty literature would suggest, but there is no evidence that such parasites have been looked for. The following list of protozoan parasites recorded from North and Central America is made up to a considerable extent of records of experimental infestations. The list covers only references readily available and is not intended as an exhaustive compilation.

Entameba zenaticum was described from dogs in the Canal Zone by Darling (1915). This protozoan was found in the colon on post-mortem examination and appears to be pathological.

Trypanosoma hippicum, described by Darling in 1910 from solipeds in the Canal Zone, has been inoculated into dogs at the same locality, according to the same author (Darling, 1913).

Trypanosoma equiperdum was reported by Mohler (1911) at Washington in a dog imported from France to this country after inoculation with the trypanosome of dourine for comparison with American strains, and American dogs were inoculated with this French strain.

Trypanosoma evansi, the cause of surra, is reported from an experimentally infected dog by Mohler, Eichhorn and Buck (1913) at Washington.

Babesia canis, the cause of canine piroplasmosis or malignant protozoan jaundice, has been reported from dogs in Porto Rico.

Spirocheta sp., an organism merely noted as "a spirochæte," is said by Gray (1913) to have been found in infective venereal granuloma of the dog in the United States by Beebe and Ewing of Cornell.

Treponema pallidum, the organism of syphilis, has been successfully inoculated in dogs by Bertarelli, and dogs may have been, and very likely have been, inoculated with this organism in the study of experimental syphilis in this country.

PROTOZOAN (?). Foster (1912) reports what he regards as an undetermined protozoan as the cause of protozoic stomatitis or sore mouth of dogs in the South. Of the mouth lesions, he says:

"Microscopic examination of sections made through these lesions, stained by Jenner's method, reveals a protozoic infection of the mucous glands, which is associated with streptococcic infection. The infecting organisms are found in the glands themselves and also in the ducts near the surface. Experiments thus far do not permit me to classify the protozoa in its proper place. . . . I find it to be actively motile, possessing a movement indicative of bipolar flagellæ, although I have not as yet stained any. It reproduces by simple fission, will grow on simple media, and presents chromatin granules. Stained from pure culture, it frequently appears as crescent-shaped on account of its movement. . . . In shape it resembles a grain of barley. Injected into the mucous membrane of a healthy subject it will produce the disease, from which it may be again obtained. It is non-sporulating."

Leishmania americana, or *Leishmania tropica americana*, the organism causing the American variety of Oriental sore, is not definitely reported from dogs in North America, so far as we are aware. However, Oriental sore has been reported from man in Panama by Darling (1911) and the disease and its parasite has been found in dogs in South America by Pedrosa, according to Laveran (1916). Laveran states:

"The cases of natural infection of dogs by *Leishmania americana* are very rare. In 1912, Pedrosa observed, in northern Brazil, two dogs that had ulcerations of the nasal mucosa. One of the dogs . . . was in bad condition; he had cutaneous ulcerations beside the lesion of the left nostril; smears made from scrapings from the nasal ulcer showed numerous Leishman bodies identical with those from the human subject. The master of the other dog had an ulcer of the foot that was diagnosed as leishmaniosis, and he made the dog lick his ulcer; the animal thereby became directly infected . . .; the diagnosis rested on the macroscopic appearance of the ulceration, and the history of the case."

Rangelia vitalii was described by Carini and Maciel, in 1914, from the blood of dogs in Brazil. It is said to resemble *Leishmania*, but lacks a visible centrosome. It causes a disease known as nambiuva, bleeding ear, yellow fever of dogs, or blood plague, characterized by icterus and cutaneous and internal hemorrhage. It has not yet been reported from North or Central America, so far as we are aware.

Neurorhynchus hydrophobicus is the name applied to the supposed protozoan organism causing rabies. However, the etiological agent in rabies is commonly regarded as not yet established. The well known Negri bodies are regarded as diagnostic of rabies, but their identity with, or relation to, the cause of rabies is still debated. Owing to the laxity in our treatment of dogs, rabies is rather common in the United States.

SUMMARY. *Diplospora bigemina* has been found in 7.5 per cent of 200 dogs examined at Detroit. The occurrence of this coccidian in man in other countries gives it considerable importance and it may be found to occur in man in this country. The coccidian is reported from the western swift fox, indicating that it may occur in the West, the home of the fox, or may occur in the East, where the foxes examined were living in a zoological garden.

Under favorable temperature conditions, oocysts in 10 per cent potassium bichromate solution appear to develop infective sporozoites in two days, but under less favorable conditions, probably more nearly those found in nature, this may require two weeks or longer. From the feeding of oocysts containing infective sporozoites to the appearance of oocysts in the feces of the dogs fed, a period of as little as 3 or 4 days to as much as 11 or 12 days may elapse. Infections are self-limited. In one animal oocysts were present in the feces for 12 days; subsequent attempts to infect this animal failed and there was apparently an acquired immunity to coccidiosis.

With infestations naturally acquired, dogs often show a practically normal digestive tract post mortem, but with heavy infestations, usually the result of feeding coccidia experimentally, hemorrhagic conditions have been found that may be related to the presence of the coccidia. The disease is afebrile, though experiments suggest that there is a slight elevation of tempera-

ture during the time that oocysts are found in the feces. Diarrhea, at times with the presence of gas or blood in the feces, appears to be, and probably is, a symptom of canine coccidiosis.

The following protozoa have been recorded in dogs in North and Central America: *Entameba venaticum*, *Babesia canis*, *Diplospora bigemina*, *Spirocheta* sp., *Neurorhynchus hydrophobiae* and an undetermined form regarded as protozoan.

The following protozoa have been reported in experimental infestation in dogs in North and Central America: *Trypanosoma hippicum*, *Trypanosoma equiperdum*, and *Trypanosoma evansi*.

Treponema pallidum, an organism only too common in man in this country, has been inoculated in dogs by Bertarelli. *Leishmania americana* has been found in man in Panama and in dogs in Brazil, and *Rangelia vitalii* has been reported from dogs in Brazil. Any or all of these forms might be found in dogs in this country, but we find no existing records.

- CASTELLANI, A. 1917. Notes on tropical diseases met with in the Balcanic and Adriatic Zones. *Jour. Trop. M. and Hyg.*, Vol. 20 (17), Sept. 1, pp. 198-202.
- COLE, LEON J., and PHILIP B. HADLEY. 1910. Blackhead in turkeys: A study in avian coccidiosis. *R. I. Exp. Sta. Bull.* 141, pp. 137-271, 11 pls.
- CRAWLEY, HOWARD. 1912. The protozoan parasites of domesticated animals. *Bu. Anim. Indust. Circ.* 194, pp. 465-498, figs. 63-75, pls. 37-42.
- DARLING, S. T. 1911. Oriental sore in Panama. *Arch. Int. Med.*, Vol. 7, May, pp. 581-597, 6 figs.
1913. The immunization of large animals to a pathogenic trypanosome (*Trypanosoma hippicum* (Darling), by means of an avirulent strain. *J. Exp. Med.*, Vol. 17, (5), pp. 582-586.
1915. Entamebic dysentery in the dog. *Proc. Med. Ass., Isthmian Canal Zone*, Vol. 6, (1), pp. 60-62.
- FANTHAM, H. B. 1916. Protozoa. In *The Animal Parasites of Man*, London, pp. 25-210, 119 figs.
- FOSTER, ALLAN A. 1912. Protozoic stomatitis, or sore mouth of dogs. *Kans. City Vet. Coll. Quart., Bull.* 36, June, pp. 872-874.
- GRAY, HENRY. 1913. Venereal diseases in the dog, rabbit, hare and fowl. In Hoare's *A System of Veterinary Medicine*, Chicago, Vol. 1, pp. 366-371.
- GUILLEBEAU, A. 1916. Parasitic occurrence of *Eimeria stiedae* in the liver of the dog. (*Schweiz. Arch. Tierheilk.*), Vol. 58, (11), pp. 596-602, 6 figs. (Not seen.)
1917. *Idem. Rec. d. Méd. Vét.*, Vol. 93, (1-2), pp. 71-73.
- HALL, MAURICE C. 1917. Apparatus for use in examining feces for evidences of parasitism. *Jour. Lab. and Clin. Med.*, Vol. 2, (5), Feb., pp. 347-353, 3 figs.
1917. Parasites of the dog in Michigan. *Jour. A. V. M. A.*, n.s. Vol. 4, (3), June, pp. 383-396.
- LAVERAN, A. 1916. American leishmaniosis of the skin and mucous membranes. *New Orleans M. and Surg. Jour.*, Vol. 68, (9), March, pp. 582-606.
- MAKHMUS, BERNARD. 1912. Clinical diagnosis of the internal diseases of domestic animals. Chicago, 259 pp., 57 figs.
- MOHLER, JOHN R., ADOLPH EICHORN and JOHN M. BUCK. 1913. The diagnosis of dourine by complement fixation. *Jour. Agric. Research*, Vol. 1, (2), Nov. 10, pp. 99-107.
- RAILLIET, ALCIDE, and ADRIEN LUCET. 1891. Note sur quelques espèces de coccidies encore peu étudiées. *Bull. Soc. zool. de France*, Vol. 16, (9-10), Nov.-Dec., pp. 246-250. (Not seen.)
- STILES, CHARLES W. 1892. Notes on parasites. II. *Jour. Comp. Med. and Vet. Arch.*, Vol. 13, (9), Sept., pp. 517-526, figs. 1-7.
- WEIDMAN, FRED. D. 1915. *Coccidium bigeminum* Stiles in Swift foxes (Habitat Western U. S.). *Jour. Comp. Path. and Therap.*, Vol. 28, (4), Dec. 31, pp. 320-323, 3 figs.

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THE INTRAVENOUS USE OF RED MERCURIC IODIDE.

BY L. W. ROWE, M.S., DETROIT, MICH.

(From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.)

The value of mercury in combating spirochetal diseases, particularly syphilis, has long been recognized, but its exhibition in the body in a satisfactory form is attended by many difficulties. The insolubility of many of the salts of mercury coupled with their toxic action upon body cells has rendered them of very little therapeutic value. The bichloride of mercury because of its comparatively greater solubility and consequent effectiveness has been used wherever possible, but its very corrosive action upon body tissues when administered hypodermically, or even upon mucous membranes when applied in a more concentrated solution, have greatly limited its use. In combination with blood serum the bichloride has been used intraspinally with some success, but the amount of bichloride which combines with the serum is not very great and a large dose is therefore necessary. The intravenous use of a satisfactory solution of the bichloride is impossible because of the great danger from embolism due to coagulation of some of the proteins of the blood.

The red iodide of mercury, HgI_2 (sometimes called biniodide), is as nearly insoluble in water as any of the salts of mercury, and for that reason is of no therapeutic importance. When combined with an equal amount of potassium iodide, a soluble compound is formed which is a very effective germicide, indicating that the characteristic action of mercury is exhibited. The efficiency of bichloride as a germicide is very great, as is indicated by the fact that its germicidal coefficient is approximately one thousand (1000), or in other words that it is about one thousand times as efficient as pure carbolic acid, yet the average results, reported by various workers, of tests of solutions of mercuric iodide in potas-

sium iodide show that this combination is fully five times as efficient as the bichloride, or 5000 times as efficient as pure carbolic acid. With this fact in mind it was thought advisable to attempt to obtain some idea concerning the possibilities of the use of mercuric iodide intravenously by determining the toxicity of this compound to animals when administered intravenously.

A search of the literature shows that Stassano and Gompel¹ have published several short articles in which they have commented upon the great germicidal activity and comparatively low toxicity of mercuric iodide and potassium iodide, but their experimental data has been largely concerned with the germicidal action and no definite comparisons could be made because of the lack of toxicity data. Lydston² in a very short report of some clinical results states that he has frequently administered red mercuric iodide dissolved in potassium iodide intravenously with no harmful effects.

The tests shown in the accompanying tables were carried out upon guinea pigs, dogs, and rabbits, in order to show the toxicity of red mercuric iodide and potassium iodide when given intravenously to animals. Two comparative tests of mercuric chloride were also made.

From data obtainable in this laboratory the toxicity of phenol (carbolic acid) administered intravenously to guinea pigs is 0.2 gm. per kg. body weight. With this additional data the following table (VI) of comparative toxicity and efficiency should be of value.

TABLE I

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED INTRAVENOUSLY TO DOGS.

Dog No.	Weight in kg.	Dose in gm. per kg.	Dil.	Total Dose in Gm.	Result	Remarks
1	8	.01	1:100	.12	Died	Dead in 2 hours
2	6.5	.010	1:50	.13	Died	Dead in 20 hours
3	7.5	.0085	1:50	.12	Died	Dead in 2 days
4	9.5	.007	1:30	.143	Died	Dead in 3 days
5	7	.004	1:50	.140	Died	Dead in 2 1/2 days
6	9	.004	1:20	.72	Died	Dead in 3 days
7	9	.0035	1:20	.63	Lived	Lived 3 weeks 4
8	9	.0030	1:20	.63	Lived	Lived 3 weeks 4
9	10	.0025	1:20	.55	Lived	Lived 3 weeks 4

M. I. D. .0010 gm. per kg. sol. of dog

¹Stassano and Gompel. *Compt. rend. Soc. de biol.*, 1913, lxxx, 12-14; *Ibid.*, 1914, lxxvii, 9-11; *Compt. rend. Acad. d. Sc.*, 1914, clviii, 1716-19.

²Lydston, G. J. *Lancet*, No. Med. Ann., 1916, lxxvi, 1446.

TABLE II

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED
INTRAVENOUSLY TO GUINEA PIGS

No.	Weight in gm.	Dose in gm. per kg.	Dil.	Dose in Cc.	Result	Remarks
1	375	.008	1:100	.30	Died	Died during night
2	395	.005		.20	Died	
3	477	.004	1:1000	1.91	Died	
4	325	.004	1:500	.65	Died	
5	315	.004		.63	Died	
6	405	.004		.81	Died	
7	359	.004		.72	Died	
8	395	.0035		.70	Died	
9	458	.0035		.80	Died	
10	478	.0035		.83	Died	
11	455	.0035		.80	Died	
12	452	.0035		.79	Died	Died 6 days after dosing
13	445	.0030		.66	Died	
14	507	.0030		.76	Died	
15	519	.0030		.78	Died	
16	519	.0030		.78	Lived	Lived 10 days +
17	436	.0030		.65	Died	
18	463	.0025		.58	Lived	Lived 10 days +
19	327	.0025	1:1000	.82	Lived	" " "
20	350	.0020		.70	Lived	" " "
21	488	.0020	1:500	.49	Lived	" " "
22	344	.0015	1:2000	1.00	Lived	" " "
23	375	.0010		.75	Lived	" " "
24	373	.0008	1:5000	1.50	Lived	" " "

M. L. D. is .0030 gm. per kg. body weight.

TABLE III

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED
INTRAVENOUSLY TO RABBITS

No.	Weight in gm.	Dose in gm. per kg.	Dil.	Dose in Cc.	Result	Remarks
1	2000	.025	1:100	5.0	Died	Dead in 1½ hours
2	1675	.015		2.51	Died	Dead in 3 days
3	1620	.010	1:50	.81	Died	Died during night
4	3150	.0080	1:100	2.52	Died	Dead in 1 week
5	1783	.0060	1:80	.86	Died	Dead in 1 week
6	2000	.0050	1:100	1.00	Lived	Lived 2 weeks +

TABLE IV

TOXICITY OF MERCURIC CHLORIDE ADMINISTERED INTRAVENOUSLY TO DOGS

No.	Weight in gm.	Dose in gm. per kg.	Dil.	Dose in Cc.	Result	Remarks
1	6	.008	1:50	2.40	Died	Died in a few hours
2	7	6		2.10	Died	Died in a few hours
3	10	5		2.50	Died	Dead in 1 week
4	9	4		1.80	Lived	Lived two weeks

M. L. D. .005 gm. per kg.

TABLE V

TOXICITY OF MERCURIC CHLORIDE ADMINISTERED INTRAVENOUSLY TO GUINEA PIGS

No.	Weight in gm.	Dose in gm. per kg.	Dil.	Dose in Cc.	Result	Remarks
1	455	.008	1:200	.75	Died	Died during night
2	493	.007		.69	Died	Died during night
3	495	.006		.59	Died	Died during night
4	572	.005		.47	Died	Died during night
5	446	.004	1:400	.72	Died	Died during night
6	451	.003		.74	Died	Died within 2 days
7	286	.0025		.72	Died	Died 3 days later
8	252	.0025		.66	Died	Died 2 days later
9	286	.0020	1:1000	.47	Lived	Observed for 1 w'k.
10	360	.0020		.72	Died	Died in 4 days
11	358	.0020		.72	Died	Died in 3 days
12	327	.0015		.49	Lived	Observed for 1 w'k.
13	368	.0015		.46	Lived	Observed for 1 w'k.
14	395	.0015		.49	Lived	Observed for 1 w'k.
15	318	.0010		.32	Lived	Observed for 1 w'k.

M. L. D. is .0020 gm. per kg

From Table VI the advantage of the use of either mercuric chloride or red mercuric iodide over the use of phenol, by comparison of toxicity and germicidal efficiency, can be readily seen. The figures in parentheses indicate the comparison between mercuric chloride and mercuric iodide alone, using the figures obtained for mercuric chloride as unity. These figures show that although red mercuric iodide in potassium iodide when given in-

TABLE VI

COMPARISON OF TOXICITY AND GERMICIDAL EFFICIENCY OF PHENOL, MERCURIC CHLORIDE AND MERCURIC IODIDE

	COEFFICIENT GERMICIDAL	TOXICITY INTRAVENOUSLY TO GUINEA PIGS	TOXICITY INTRAVENOUSLY TO DOGS
Phenol	1	1	no data
Mercuric chloride	1000 (1)	100 (1)	.005 gm. per kg. (1)
Mercuric iodide in KI	5000 (5)	66% (%)	.004 gm. per kg. (1 1/4)

travenously is very little if any more toxic (in the case of guinea pigs it is less toxic) than mercuric chloride, yet its germicidal efficiency is five times as great as that of mercuric chloride. It should consequently be greatly preferred to the bichloride if only because of its greater efficiency.

Another factor which is worthy of serious consideration is that in all the intravenous injections into animals of mercuric iodide (some of the injections being made rapidly) not one of the animals died suddenly following the injection. This would indicate that a solution of red mercuric iodide in potassium iodide has no very strong tendency to coagulate any of the constituents of

the blood when introduced directly into the blood stream and thereby cause sudden death from embolism. On the other hand according to our observations, solutions of mercuric *chloride* have several times produced sudden death when administered intravenously to animals, indicating that an embolus is often formed. This is very apt to happen if the injection is made rapidly.

Solutions of mercuric iodide like those of most mercury salts cause marked local irritation when administered subcutaneously or intramuscularly, so that the iodide will probably never become very popular for such methods of administration.

However, with the increase of our use of intravenous therapy because of advantages which it possesses in many instances and particularly because the most successful method of combating a severe systemic disease such as syphilis is by intravenous injection of such agents as salvarsan, etc., in solution, it seemed advisable to contribute the above tables of data to our knowledge of the pharmacologic action of such an important germicide as red mercuric iodide.

It is not the object of this paper to assure physicians of the safety of the intravenous use of this salt of mercury because this can not be assumed from animal experimentation alone. It is intended merely to point out certain therapeutic possibilities as indicated by animal tests. If these can be substantiated by carefully conducted clinical tests, as it is hoped they can be, the purpose of the article will have been attained.

SUMMARY.

Red mercuric iodide in combination with an equal amount of potassium iodide can be injected in solution into animals intravenously with comparative safety if reasonable care is exercised in the manner of injection and in the size of the dose injected.

It is very little if any more toxic than mercuric chloride, safer for intravenous use, and because of its greater germicidal efficiency should be found to be of therapeutic value.



**Studies from the Research Laboratory.
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THE CULTIVATION OF MEDICINAL PLANTS.

**Third of a Series of Papers on Drug Plant Cultivation—A Symposium
in Which Some Well Known Growers Seek to Correct Certain
Current Misinformation Concerning the Subject—
Some Words with the Amateur Agriculturist.**

BY OLIVER ATKINS FARWELL.

Now that we have entered the European war, the attention of the nation is strongly directed to the intensive and extensive production on a large scale of all the necessities of life and of those other things essential to the successful prosecution of the war. Under these conditions, it is very natural that many of those connected in any way with the medical and allied sciences should turn their attention to the cultivation of medicinal drugs.

The impression has become general that easy fortunes can be made by the cultivation of medicinal plants in back yards or on an acre or two of unused land. Let me say, once and for all, that this cannot be done.

A KNOWLEDGE OF AGRICULTURE IS REQUIRED.

The inexperienced person cannot hope to grow drug plants successfully. He will make a failure of it. He cannot cultivate, side by side, drug plants that are natives of the tropics, the temperate zones, and the colder regions of high altitudes. Some species cannot be grown anywhere in this country. He cannot cultivate drug plants in soils which are not conducive to large yields of their respective constituents and expect to market them; therefore, he must have some knowledge of plant constituents so that he may know whether his land will produce in the plant cultivated those peculiar properties which make it valuable as a therapeutic agent. Some species require but little gardening knowledge and may be grown as readily as wheat, turnips, and other ordinary garden crops, while others require the skill and practical knowledge of an experienced agriculturist. As the time

of gathering and the method of handling are prime factors in the production of first-class commercial drugs he must know *when to gather* and *how to dry* them. Different parts are gathered at different seasons, and the harvested drug, bark, leaves, and roots should be dried as quickly as possible without sweating, usually in some open room or in some spot where there is a free circulation of air.

Market conditions encountered by the drug plant grower in disposing of his crops are peculiar, require careful study, and should be considered by the amateur. It has been said that there are not more than 3,000 plants that have been used at one time or another the world over as therapeutic agents. In this country probably about 500 species are used by manufacturing pharmacists, and the yearly consumption of each may range from a few pounds of some little used species to 2,000,000 pounds or more of cascara sagrada. Nearly all of these drugs grow profusely in a wild state and in sections where labor is cheap and the cost of gathering is at a minimum. The market value of such drugs is so low that their cultivation for commercial profit at the present time is entirely out of the question. Others are cultivated in Europe and Asia, where women and children constitute the principal part of the labor and work for little or nothing, thus rendering the cost of the imported article so low that cultivators of the same drug in this country are unable to find a profitable market for their product.

Under normal conditions, digitalis, which was formerly widely cultivated in Europe, could be imported more cheaply than the wild-grown drug of the Pacific coast States could be gathered. The outlet for most medicinal plants is limited, and unless the movement for cultivation is intelligently directed from some central source the market will soon be glutted, resulting in wasted efforts and financial loss, principally to those who could little afford it. Before venturing on the cultivation of medicinal plants the farmer or individual should first determine whether or not the land to be cultivated will produce a more valuable crop of some other staple. He must determine this for himself. Many drug plants require a large amount of hand labor, which will make the cost of production high, and to this cost must be added that of packing and transportation.

The demand for crude drugs, as compared with that for other crops, is *very light*; two facts are, therefore, brought prominently into view if the cultivation is to be financially successful, viz.: Cultivators must be few and intelligently directed; and the cultivation of only a few plants with *high market values* will insure a profitable return.

I would suggest the following species as those that will bring sufficient returns to repay the investment in time and money spent on their cultivation. The seed probably can be obtained from the leading seedmen and nurserymen of the country.

BELLADONNA.

This drug is derived from the solanaceous plant *Atropa Belladonna* Linn. It is a native of Europe, and is one of a group of mydriatic plants of the potato family (Solanaceæ). Atropine, the active principle, is found in all parts of the plant, so all parts should find a ready market. It is a herbaceous plant, two or three feet high, and with an equal spread of foliage. The flowers are cylindrical and of a purplish color, the berry is nearly globular, one-half inch or so in diameter, deep-violet, sweetish, and very poisonous. It withstands the cold winter climate of States as far north as Michigan very well, and probably can be grown successfully wherever potatoes are grown.

It may be self-sown, but spreading in this manner is not usual. The seed may be sown or drilled in the usual way in rows three feet apart, using from two to three pounds of seed to the acre. Fall sowing is best, but spring sowing may also be made, and, as many of the seeds remain dormant or are winter killed, it will be necessary to sow seed in boxes during the winter under glass to produce a reserve of plants to fill in the gaps due to these losses. If seed is sown in the field in the autumn or spring, a top dressing of manure will be beneficial in protecting the young seedlings from injury through sudden and pronounced changes of temperature or frosts. In my experience the method of sowing seed in the field has not proved satisfactory, and I would recommend the method of germinating seed under glass during the winter and transplanting in the spring. An ounce of seed if properly handled should produce enough plants to cover an acre of ground. It should be sown in pots or boxes, under glass, in midwinter, and

when the seedlings are sufficiently large, transplanted to single pots or to boxes and placed about two inches apart, much as tomatoes, cabbages, etc., are handled. These plants should be transferred to the field in May while the ground is still moist and soft from the spring rains, the young plants being placed about eighteen inches from each other in rows three feet apart. But at the close of the first year every other plant should be taken up in order to provide ample space for the larger and stronger plants



Harvesting Belladonna.

Courtesy of Stafford Allen & Sons, Limited, of London

of following years. The roots thus taken up can be transplanted, thus doubling the size of the field that can be marketed.

The crop of leaves for the first year will be light and may be harvested in September by breaking off the tops of the plants. Two crops can generally be gathered in subsequent years, the first in June, during the flowering season, and the second in September or October, just before the leaves begin to fade. Stems may

be harvested at the close of the year and marketed for the production of the alkaloid, atropine. At the close of the fourth year the roots may be harvested, as the plants are not liable to yield either stems or leaves of much value after that time.

The harvested leaves and roots should be kept in a shady spot while being dried, or in some cool, dry room, with ample circulation of air. Roots should be split or sliced to facilitate the process. They should be dried quickly and should not be permitted to "sweat," as that renders them useless. The leaves will lose about four-fifths of their weight and the roots about two-thirds. An acre should produce one ton or a little more of dry leaves in the second and succeeding years.

HENBANE.

This plant, the *Hyoscyamus niger* Linn., belongs to the same group of plants as belladonna, and its active principle is very similar to, if not identical with, atropine. Like belladonna, it is a native of Europe. It is a biennial, however, instead of a perennial and this necessitates its collection but once in two years. The leaves and flowering tops are the only parts used, and these lose four-fifths their weight in drying. The cultivation is much the same as that of belladonna. As the plants are much smaller than belladonna they can be set two feet apart each way. The time for gathering is from June to August.

Belladonna may be attacked by insects, but, as a rule, it is not disturbed. On the other hand, henbane is attacked as ravenously as is the potato. Since the leaves are the part used for medicinal purposes, the use of arsenical insecticides to destroy the beetles and their larvæ is impossible, and dusting with lime or the use of some non-poisonous emulsion, or hand-picking of eggs, larvæ, etc., are the only methods available.

DIGITALIS OR FOXGLOVE.

Foxglove, *Digitalis purpurea* Linn., is, like the foregoing, a native of Europe. The leaf is the part used, and the activity lies in the non-nitrogenized neutral principle digitalin, which is a powerful heart sedative. The plant prefers a siliceous soil, but will do well on a calcareous one. It is usually a biennial and the leaves are hand-picked from the flowering plant during the summer of

the second year. The seed is very small and should be thoroughly mixed with fine sand to insure its even distribution when sown. Its germination is very uncertain, and it should be but thinly covered with soil. Two pounds of seed should be sufficient to sow an acre. Its cultivation is similar to that of an ordinary garden plant and it does well in partial shade, but prefers a moderate amount of sun. An acre should produce from one-fourth to one-half a ton of dried leaves.

HYDRASTIS OR GOLDEN SEAL.

Golden seal, *Hydrastis Canadensis* Linn., is a native of eastern North America. The great demand for the drug for manufacturing purposes has practically exterminated the wild plant and cultivation is extremely difficult because of the difficulty in germinating the seed and of producing conditions similar to those of its native habitat. It grows naturally in wet, rich woods, where it is not subject to the influences of the hot sun, dry winds, or excessive rainfall. These conditions may be approximated by making the beds in orchards or other places where the shade of trees will cover them during the hottest parts of the day. The beds should be prepared with plenty of leaf or peat mold, so as to imitate natural soil conditions, and should be remulched every year. They should be kept moist and sufficiently well drained as to prevent waterlogging. If there is no natural shade to cover the beds, artificial shade can be produced by a frame of latticework built over them. Seed can be germinated if sown in fine potting soil or leaf mold and covered slightly. The best way is to get the fresh rhizome and divide it into as many sections as it has buds, planting a section every six inches in rows one foot apart. In transplanting, care should be taken not to tread on the growing rows or beds. The rhizomes can be taken up and divided each spring (in April or May). After three years, harvesting may begin; the larger rhizomes and roots being set aside for proper preparation for marketing and the smaller ones divided and reset. It has been claimed that an acre will produce from 1000 to 1500 pounds of dried roots. The stems and leaves also have a marketable value. They are usually collected after the fruit matures and just before they begin to fade.



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THE DETERIORATION OF DIGITALIS EXTRACTS.

BY HERBERT C. HAMILTON.

(From the Research Laboratory of Parke, Davis & Co., Detroit Mich.)

A recent publication on this subject by Pittenger and Mulford Jr.¹ is so revolutionary in character that while it would require a rather long series of experiments to cover the ground fully, a preliminary note on the subject seems called for because these results would lead one to infer that the tincture is practically worthless.

It is a well-known fact that digitalis has not yet been prepared in any absolutely stable form adapted to clinical use. The reason for this instability is still a matter of conjecture. Bourquelet² and Choay³ recognizing the presence of enzymes in the fresh leaves considered them to be the cause of the deterioration, because of enzyme action on the glucosides. The remedy suggested was that of exposing the crude drug before drying to the action of strong alcohol vapors which killed the enzymes, dehydrated the drug and hastened the drying process, all of which are undoubtedly valuable but also more or less impracticable steps. That this is not the remedy, however, is demonstrated by the deterioration of the fluidextract, the tincture and the extract which have been extracted with alcohol strong enough to kill or to precipitate any enzymes present in the drug.

Some experiments in this laboratory carried over a number of years indicate that the presence of certain constituents of unknown character are largely but not entirely responsible for the observed instability. This, however, is reserved for publication later.

The object of this preliminary note is to call attention to data either already published or accumulated through years of close

association with the extraction and testing of digitalis and to pave the way for a further publication of fresh data bearing directly on the point at issue, namely, whether digitalis is really as unstable as these results would indicate. If it were true that this valuable agent is so unstable, in many cases it would be practically worthless before it reaches the shelves of the druggist. This however is inconceivable since clinically the tincture is considered as valuable as any of the digitalis preparations.

Summarizing the data submitted by Pittenger and Mulford Jr., their results are shown in the following table:

Menstruum	50% alcohol.	50% alcohol.	80% alcohol.
Character of drug.....	Not defatted.	Fat-free.	Fat-free.
Average loss on 5 samples in 8 months...	47.8%	22.8%	40.7%

NOTE: These percentages of loss are based on the original assay.

From this one might conclude that a tincture is of little value unless made by extracting fat-free leaves with 50 per cent alcohol, since the fat-free tincture with 80 per cent alcohol is apparently no more stable than that with less alcohol. It seems improbable, however, that either the higher percentage of alcohol or the absence of fats is responsible for the great loss in the third series.

Hale⁴ found the official fluidextracts, which are not fat-free, to have lost only an average of 6.6 per cent in two years.

Roth⁵ observed an average loss of activity in 7 samples of fat-free tinctures of digitalis of 14 per cent in 6 months. Of this number two showed no loss, while two others suffered an exceptionally high loss.

Houghton and Hamilton⁶ published the results of a series of experiments on deterioration which was summarized as follows:

LOSS OF POTENCY OF DIGITALIS PREPARATIONS WITH AGE.

	No. sample.	Av. No. per cc. when mfg.	H. T. U.'s. when mfg.	Years later.	Av. No. H. T. U.'s.	Av. yearly loss, %.
Prep. Extract.....	11	260		5	160	8
Fl. Ext. U.S.P. 7th Rev. .	8	72		6	55	4
Fl. Ext. U.S.P. 8th Rev....	11	55		3½	35	10
Tincture U.S.P. 8th Rev...	8	7		8	5	9

From the results in the above table and some other data not included the authors concluded:

1st. That a maximum average loss of 10 per cent a year can be expected in tinctures or fluid extracts of digitalis.

2nd. That an alcoholic content of more than 50 per cent in the extracting menstruum not only more completely extracts but also more nearly preserves the activity of digitalis.

This has been demonstrated so conclusively that the 9th Revision of the U. S. P. eliminates the low alcoholic menstruum and some manufacturers discarded it after only a short trial.

It seems improbable therefore that tinctures with this low alcoholic content would be uniformly found more stable than when extracted with 80 per cent alcohol whether the drug was fat-free or not. One cannot avoid the thought that perhaps each of the investigators quoted obtained exceptional results due to certain unusual conditions; further, that no data either good or bad should be accepted as representing the average condition of digitalis after any particular period of aging.

Goodall⁷ summarizes some results of his as follows:

"Tincture of digitalis probably retains its full activity for one year, but after that period deterioration of its potency to an important extent is likely to take place." This sounds like a reasonable conclusion, which, however, may not be verified by any single set of experiments.

The following data was obtained by my colleague, L. W. Rowe, from retests of six tinctures after an average period of six and one-half months. The tinctures were in every case extracted with 70 per cent alcohol and in two cases fat-free drug was used.

These assays were carried out by the M. L. D. Frog-heart Method.⁸

SUMMARY OF ASSAYS OF TINCTURE OF DIGITALIS.
(U. S. P. MENSTRUUM.)

Number.	Drug.	1st test.	2nd test.	Per cent loss.	Age.
A	Fat-free	St'd	90	10	8 mo.
B	Not defatted	110	90	19	7½ mo.
C	Not defatted	200	130	35	5½ mo.
D	Not defatted	160	130	19	5 mo.
E	Fat-free	St'd	St'd	0	8 mo.
F	Not defatted	140	80	43	6 mo.

Average loss on 6 samples—21 per cent.

Average loss on fat-free samples—5 per cent.

Average loss on other samples—29 per cent.

The preceding data show very clearly that:

1st. The degree of deterioration varies with different lots.

2nd. The fat-free tincture made with 70 per cent alcohol—two

out of six samples—is apparently less subject to deterioration than that from the original drug.

3rd. The deterioration of tincture of digitalis is not so uniformly rapid as isolated experiments would indicate.

BIBLIOGRAPHY.

- ¹Pittenger and Mulford, Jr., J. A. PH. A., March 1918, p. 236.
- ²Bourquelet, *Jour. de Pharm. et de Chémie*, 1911, p. 149.
- ³Choay, *Jour. de Pharm. et de Chémie*, 1911, p. 343.
- ⁴Hale, *Hygiène Laboratoire*, Bulletin No. 74.
- ⁵Roth, *Hygiène Laboratoire*, Bulletin No. 102.
- ⁶Houghton and Hamilton, *Am. Jour. of Pharmacy*, Oct. 1909.
- ⁷Goodall, *Br. Med. Journal*, 1912, Vol. I, p. 887.

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**STUDIES RELATIVE TO THE APPARENT CLOSE
RELATIONSHIP BETWEEN BACT. PERTUSSIS
AND B. BRONCHISEPTICUS.***

I. CULTURAL AGGLUTINATION AND ABSORPTION REACTIONS.

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Mallory and his associates in 1912 and 1913, while attempting to prove the relationship of *Bact. pertussis* to whooping cough by animal inoculations, found that the problem was much more complicated than anticipated, their interpretations being clouded by the introduction into the question of the *Bacillus bronchisepticus*, as a result of its presence in some of the animals used for experimental purposes.

During the discussion of the paper by Mallory the observation was made by Dr. J. L. Rhea, that the lesions in pertussis in the human being, due to the bacterium of Bordet, are similar to the lesions in the dog, which result from an infection with *B. bronchisepticus*, the cause of distemper, and that this fact suggested an interesting relationship between the two organisms. Later, Mallory states:

Further experimental work is evidently needed in order to clear up the subject. The two organisms closely resemble each other morphologically and in cultures on potato blood agar, but can be distinguished by their difference in motility and their alkali production in litmus milk.

Soon after the appearance of the work of Mallory, the writers started some experimental work with the two organisms in ques-

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tion, to determine, if possible, just how close was this relationship which apparently existed between them.

At first the experiments were undertaken with two strains of *Bact. pertussis* which had been cultured since 1911, one having been furnished by the laboratory of Bordet and the other isolated in our own laboratory, and three strains of *B. bronchisepticus* isolated by one of us (N. S. F.), one from a dog in 1908, one from a monkey in 1912 and one from a human subject in 1913. Later on these strains were augmented by ten strains of *Bact. pertussis*, furnished by Dr. Olga R. Povitzky of the New York Board of Health Laboratory, through the courtesy of Dr. Park, and three strains of *Bact. influenza*, together with one strain of a *Bact. pertussis*-like organism from pertussis sputum, isolated in our own laboratory.

CULTURAL REACTIONS.

When first isolated, the *Bact. pertussis* develops slowly and, as a rule, preferably on special media, as reported by Bordet, Woolstein and others. After several months of repeated transplantings, however, its ability to grow on various media gradually increases until it finally presents a growth almost identical to, and nearly as luxuriant as, that of *B. bronchisepticus*, and by that time can be cultured on ordinary media.

It has been found by the writers that the one great difference between the two organisms lies in their power of locomotion: the *B. bronchisepticus* is motile while the *Bact. pertussis* is non-motile, several months of attempting to develop a strain that would give some evidence of motility resulting in failure.

While the cultural reactions have been found practically identical, even to the alkali production in litmus milk, contrary to the report of Mallory, and the tan color on potato is shared by both, yet, with the *Bact. pertussis*, these reactions are extremely tardy in making their appearance, usually taking about two or three weeks longer than with the *B. bronchisepticus*. At the end of this time, however, it is practically impossible to differentiate between the cultures of the two organisms.

The following outline will show the characteristics of these organisms, in a general way:

	B. BRONCHISEPTICUS	BACT. PERTUSSIS
Morphology.....	Very small, slender rod, showing bipolar staining	Small, slender rod, showing marked bipolar staining
Gram.....	Negative	Negative
Motility.....	Motile	Non-motile
Agar slant.....	Translucent, filiform growth	Translucent, filiform growth
Bouillon.....	Cloudy. Older growth with heavy, stringy sediment	Cloudy. Older growth with heavy, stringy sediment
Potato.....	Tan. Light tan in twenty-four hours to dark tan in three weeks; medium becoming tanned	Tan. Light yellow in twenty-four hours to tan in 3-5 weeks; medium tanned.
Litmus milk.....	Alkaline. Slightly blue at the surface in forty-eight hours. This color proceeds downward, becoming very dark greenish blue in about seven days, while the lower part decolorizes	Alkaline. In about 6 days the litmus milk begins to decolorize at the dark bottom of the tube, becomes slightly blue in upper portion in four weeks, and in from eight to ten weeks can scarcely be distinguished from <i>B. bronchisepticus</i> .
Litmus-lactose agar.....	Alkaline (forty-eight hours)	Alkaline (four to six days)
Glucose agar.....	No gas	No gas
Gelatin.....	Not liquefied	Not liquefied
Indol.....	Negative	Negative
Nitrites in nitrate broth...	Negative	Negative

AGGLUTINATION REACTIONS.

The agglutination reactions of these two organisms have presented some very interesting and rather novel phenomena which, to the writers, suggest at least a distant relationship between them.

In the early part of this year Povitzky and Worth reported the results of some agglutination experiments with these organisms, using a *Bact. pertussis* antiserum only, and concluded that,

B. pertussis strains can be specifically identified from hemoglobinophilic bacilli, pertussis-like bacilli and *B. bronchisepticus*. In no instance was there cross agglutination between these organisms—at least not higher than 1:40.

Our work has corroborated that of the authors so far as they have gone. Table 1 gives the results of agglutination between antipertussis serum and suspensions of *Bact. pertussis*, a per-

TABLE 1

Agglutination tests between antipertussis serum and heterologous suspensions.
Results August 31, 1916. Serum from rabbit 7, treated with Bact.
pertussis no. 0363 (Bordet)

DILUTIONS	SUSPENSIONS OF								
	Bact. pertussis		B. bronchisepticus			Pertussislike bacteria	Bact. influenzae		
	No. 0363 (Bordet)	No. 0590 (Ferry)	No. 36 (Dog)	No. 123 (Monkey)	(Human)		No. 032558	T. b No. 2	"S."
1-10	-	-	-	-	-	+	-	-	+
1-20	-	-	-	-	-	-	-	-	-
1-40	-	+	-	-	-	-	-	-	-
1-80	+	++	-	-	-	-	-	-	-
1-200	+++	+++	-	-	-	-	-	-	-
1-400	+++	+++	-	-	-	-	-	-	-
1-800	+++	+++	-	-	-	-	-	-	-
1-1600	+++	+++	-	-	-	-	-	-	-
1-2000	+++	+++	-	-	-	-	-	-	-
1-3200	+++	+++	-	-	-	-	-	-	-
1-6400	++	+++	-	-	-	-	-	-	-
1-10000	+	++	-	-	-	-	-	-	-
1-20000	-	+	-	-	-	-	-	-	-
1-40000	-	-	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-

This table and the following one illustrate the presence of pro-agglutinoids in *Bact. pertussis* and *B. bronchisepticus* antiserum making it necessary to test all normal and immune sera in dilutions higher than 1-80.

tussis-like bacillus, *B. bronchisepticus*, and three hemoglobinophilic bacilli. But on using a *B. bronchisepticus* antiserum, the results are entirely different, as the *Bact. pertussis* suspensions agglutinate nearly as well as the homologous suspension, the *B. bronchisepticus*. The results of such a test are given in table 2, where agglutination tests were made with suspensions of fourteen strains of *Bact. pertussis* and a homologous suspension of *B. bronchisepticus* against antibronchisepticus serum. And finally,

table 3 is a summary of all the homologous and cross agglutination tests.

The results therefore show that the *B. bronchisepticus* antiserum will agglutinate both the *B. bronchisepticus* and *Bact. pertussis* antigens, while the *Bact. pertussis* antiserum will agglutinate only its homologous antigen, the *Bact. pertussis*. This reaction was characteristic of every strain of *Bact. pertussis* and *B. bronchisepticus* under observation.

Whether this shows a true relationship between the two organisms, and can be called a specific reaction, is a question.

Preparation of the antigens for the production of the antisera. *B. bronchisepticus* was grown on plain agar, *Bact. pertussis* No. 0363 (Bordet) and 0590 (Ferry) on ascitic agar, and the New York strains of *Bact. pertussis* and the hemoglobinophilic bacilli on whole-blood (rabbit) agar. Twenty-four-hour growths were washed off in 0.2 per cent trikresol in physiologic salt solution—1 cc. to a culture of *Bact. influenza*, 2.5 cc. to a culture of *Bact. pertussis* and 5 cc. to a culture of *B. bronchisepticus*. The suspensions were thoroughly shaken in a mechanical shaker and, after two days, tested for sterility.

Production of antisera. Before being treated, the serum of each rabbit was tested for agglutinins against all of the organisms under discussion. Any animal showing an agglutination higher than 1-20 against *B. bronchisepticus* antigen or 1-40 against any of the other organisms, was not used.

The rabbits were given three intravenous injections of increasing doses from 0.5 cc. to 2 cc. three days apart and were bled on the fourth day after the last dose; 0.2 per cent trikresol was added to the serum to insure sterility.

Preparation of suspensions for agglutination tests. The suspensions were prepared in general, as follows:

Each culture was transplanted daily for from three days to three weeks—depending upon the organism—on media best suited to it, to insure a good vigorous growth. Then twenty-four-hour cultures of these were planted on plain agar in quart whiskey flasks—except in the case of *Bact. influenza*, for which whole-blood agar was used. It has been found advantageous to use agar in whiskey flasks, as this method not only gives a larger amount of suspension for less labor, but it also gives a far heavier and healthier growth than when tubes are used, probably because of the greater supply of media, moisture and air. An abundant growth of *Bact. pertussis* can be obtained on plain agar in flasks when it will grow only slightly or not at all on plain agar in tubes. And a young, vigorous growth is necessary to the production of homogeneous suspensions. Also in using agar without either ascitic fluid or blood, all possibility of clumping from this source is avoided. In the case of the influenza bacillus it is necessary to use blood agar to obtain any growth. Just

enough whole blood is added to agar to insure growth and it is used before any hemolysis takes place, in order that the suspension may contain as little blood as possible.

The flasks were incubated for from eighteen to twenty-four hours and the growth washed off with 0.5 per cent formalin in physiologic salt solution. The suspensions were then shaken for a few hours, and later, after being tested for sterility, were filtered through paper and standardized to about 2000 million per cubic centimeter.

With this technique, homogeneous suspensions of all the organisms used were produced.

Agglutination tests. In carrying out the tests, the serum was diluted with physiologic salt solution and each tube contained 0.5 cc. suspension plus 0.5 cc. diluted serum. The tests were all macroscopic and were incubated at 37°C. for twenty-four hours. (+ + +) represents complete agglutination with fluid clear; (+ +) partial agglutination with marked clumping, but fluid not entirely cleared up; (+) slight agglutination, but still with positive clumping; and (—) no clumping, no clearing.

Specially graduated 1 cc. pipettes were used for making the serum dilutions and a different pipette was used for each dilution. All glassware used in connection with the tests was clean and sterile.

AGGLUTINATION REACTIONS WITH SERUM FROM DISTEMPER RABBITS AND DOGS.

In testing apparently normal rabbits for agglutinins, before beginning inoculation for the production of antisera, we found that if a serum agglutinated *B. bronchisepticus* in a dilution higher than 1-20, it also agglutinated *Bact. pertussis* and generally in higher dilutions. In one instance when there was no agglutination against *B. bronchisepticus*, the serum agglutinated *Bact. pertussis* in a dilution of 1-400 (rabbit E) (see table 4).

A few of these rabbits subsequently developed symptoms of distemper; the others may have recovered from an attack.

Sera from only two distemper dogs have been tested, but, with these, similar results were obtained. As in rabbits, agglutinins for *Bact. pertussis* were manifest in higher dilutions than for *B. bronchisepticus*. These dogs exhibited typical symptoms of distemper and were in the later stages of the disease.

Dog 1 agglutinated *B. bronchisepticus* No. 36 (dog) at 1-20; and *Bact. pertussis* No. 0363 (Bordet), 1-400, and No. 93, 1-1000. Dog 2 agglutinated *B. bronchisepticus* No. 36 (dog) at 1-80; and *Bact. pertussis* No. 0363, 1-1000, and No. 93, 1-1000.

On absorption with *B. bronchisepticus* the agglutinins for that organism are removed, while the agglutinins for *Bact. pertussis* are affected little or not at all. On absorption with *Bact. pertussis*, the pertussis agglutinins are removed, while those for *B. bronchisepticus* are unaffected.

ABSORPTION REACTIONS.

Upon submitting to absorption tests those antisera which were produced by the injection of *B. bronchisepticus* antigen into rabbits, and which were found to agglutinate both the *B. bronchisepticus* and *Bact. pertussis* suspensions, the following results were obtained.

Upon absorbing with *B. bronchisepticus* suspension, the agglutinins for *B. bronchisepticus* (the major agglutinins) were absorbed, but the *Bact. pertussis* agglutinins (the minor agglutinins) were still intact (table 5), and an absorption with *Bact. pertussis* was necessary before they were neutralized. In other words, the *B. bronchisepticus* antigen stimulated the formation of both *B. bronchisepticus* and *Bact. pertussis* agglutinins, but contrary to what one would expect, was not able to absorb the *Bact. pertussis* agglutinins (the minor agglutinins). It was also found, in the case of dogs suffering with distemper, that the serum agglutinated *Bact. pertussis* antigen in higher dilutions than the *B. bronchisepticus* antigen. Absorption with *B. bronchisepticus* antigen took out only the *B. bronchisepticus* agglutinin, while it was necessary to absorb with *Bact. pertussis* antigen before the *Bact. pertussis* agglutinin was neutralized (see table 7). The *Bact. pertussis* agglutinins, therefore, were fixed or stable, as far as the *B. bronchisepticus* was concerned, but absorbable for the *Bact. pertussis*, and this type of an agglutinin, which can be produced by one antigen to be taken up or absorbed more readily by another, has been called by the writers, for the lack of a better term, a transitive agglutinin.

The antisera were identical to those used for the agglutination tests.

The heavy suspensions used for absorption were made from twenty-hour growths on plain agar in whiskey flasks, suspended in .85 per cent salt solution to which had been added 0.5 per cent formalin. About 10 cc.

salt solution was used to a flask for *B. bronchisepticus* and 4 cc. for *Bact. pertussis*. The suspensions were shaken over night in a mechanical shaker and then strained through mull. Each strain had been transplanted daily for several days before using so that very heavy growths were obtained.

Absorption tests. Equal parts of heavy suspension and serum were mixed; incubated at 37° C.; then centrifugalized and the supernatant

TABLE 5

Serum from rabbit 1, treated with B. bronchisepticus absorbed (1-40) with B. bronchisepticus

DILUTIONS	AGGLUTINATION			
	Before absorption		After absorption	
	B. bronchisepticus No. 36	Bact. pertussis No. 0590	B. bronchisepticus No. 36	Bact. pertussis No. 0590
1-80	+++	+++	++	+++
1-200	+++	+++	-	+++
1-400	+++	+++	-	+++
1-800	+++	++	-	++
1-1600	+++	-	-	-
1-2000	+++	-	-	-
1-3200	++	-	-	-
1-6400	+	-	-	-
1-10000	-	-	-	-
Control	-	-	-	-

fluid tested for agglutinins. The time of incubation, the number of absorptions and the dilution of the serum were varied in a number of ways. For example, we found that when either antipertussis or anti-bronchisepticus serum was diluted to 1-40, we obtained a more nearly complete absorption than when diluted 1-10, which may be due to the fact that complete agglutination does not occur in the lower dilutions with either of the organisms, especially with *Bact. pertussis*. And in the absorption tests, it was noted that clumping and clearing were not so complete at 1-10 or 1-20 as at 1-40.

Again, serum from rabbit 5, treated with *B. bronchisepticus* (Human), was absorbed as many as four times, the dilutions being from 1-5 to 1-40 and the tests being shaken in a mechanical shaker before each incubation, with no effect on the agglutinins for *Bact. pertussis*.

It was found that when antibronchisepticus serum was absorbed with *B. bronchisepticus* sufficiently to remove the agglutinins for *B. bronchisepticus* the agglutinins for *Bact. pertussis* were still unaffected. This could be done by an absorption at 1-40, incubated 24 hours. Table 5 gives the results of such an experiment.

But it required repeated absorption with *B. bronchisepticus*

before any marked effect was produced in the pertussis agglutinins, and this happened only with the dog strain.

Finally, the following method was used with the six antibronchisepticus sera. The results of these experiments are given in table 6.

TABLE 6
Absorption tests with B. bronchisepticus and Bact. pertussis antisera

SERUM	ABSORBED WITH	AGGLUTINATION AFTER ABSORPTION	
		B. bronchi- septicus	Bact. pertussis
Antibronchisepticus.			
No. 36 (dog), rabbit 1..	(Original titre)	1-6400	1-100
	B. bronchisepticus no. 36	—	—
	Bact. pertussis no. 0590	1-6400	1-80
No. 36 (dog), rabbit 2..	(Original titre)	1-10000	1-400
	B. bronchisepticus no. 36	—	—
	Bact. pertussis no. 0590	1-10000	—
No. 123 (monkey), rabbit 3	(Original titre)	1-10000	1-3200
	B. bronchisepticus no. 123	—	1-2000
	Bact. pertussis no. 0590	1-10000	1-80
No. 123 (monkey); rabbit 4	(Original titre)	1-20000	1-3200
	B. bronchisepticus no. 123	—	1-80
	Bact. pertussis no. 0590	1-6400	—
Human, rabbit 5.*...	(Original titre)	1-6400	1-800
	B. bronchisepticus (Human)	—	1-800
	Bact. pertussis no. 0363	1-6400	—
Human, rabbit 6	(Original titre)	1-10000	1-800
	B. bronchisepticus (human)	—	1-800
	Bact. pertussus no. 0363	1-10000	1-80
Antipertussis			
No. 0590, rabbit 10	(Original titre)	1-10	1-10000
	Bact. pertussis No. 0590	—	—
	B. bronchisepticus No. 36	—	1-6400

Each serum was absorbed three times with its homologous antigen—that is, serum from rabbit treated with *B. bronchisepticus* (dog) was absorbed with the dog strain—and with *pertussis* antigen as follows:

First absorption, serum 1-10 incubated two hours.

Second absorption, serum 1-20 incubated two hours.

Third absorption, serum 1-40 incubated eighteen hours.

TABLE 7

Absorption tests with serum from distemper dogs

SERUM	ABSORBED WITH	AGGLUTINATION AFTER ABSORPTION		
		B. bronchi- septicus no. 36 (dog)	Bact. pertussis	
			No. 0363	No. 93
Dog 1.....	(Original titre)	1-20	1-400	1-1000
	B. bronchisepticus no. 36	—	1-200	1-1000
	Bact. pertussis no. 0363	1-20	—	1-20
Dog 2.....	(Original titre)	1-80	1-1000	1-1000
	B. bronchisepticus no. 36	—	1-800	
	Bact. pertussis no. 0363	1-80	—	

SUMMARY AND DISCUSSION.

1. After repeated transplantings *Bact. pertussis* has been found to give the same cultural reactions as *B. bronchisepticus*—the tan growth on potato and the alkaline reaction in litmus milk being the most prominent characteristics. The difference in motility and the tardiness in the reactions of *Bact. pertussis* on culture media are differential characteristics.

2. *B. bronchisepticus* antiserum agglutinates not only *B. bronchisepticus* (1-6400 to 1-2000), but also *Bact. pertussis* (1-400 to 1-6400).

3. *Bact. pertussis* antiserum on the other hand agglutinates only the pertussis bacillus.

4. There was no cross agglutination between *B. bronchisepticus* and a pertussis-like bacillus or three hemoglobinophilic bacilli. Also, there was no agglutination between *Bact. pertussis* and these organisms.

5. *Bact. pertussis* antiserum of high agglutination titre (1-3200 to 1-20,000) has been produced, in rabbits, by three intravenous inoculations (three days apart) of sterile, unheated vaccines of fifteen strains of *Bact. pertussis*.

Povitzky and Worth, in the article cited above, conclude that,

A strongly agglutinating pertussis serum was best obtained in the rabbit by ten to twelve intraperitoneal inoculations of living cultures at seven-day intervals. Agglutinins are also produced by vaccines, but not as abundantly as by living cultures.

After ten to twelve inoculations of live cultures, their rabbits show a titre of from 2400 to 5000, and one rabbit went to 10,000.

Over 50 per cent (9 out of 17) of our rabbits show a titre of 20,000, and only two were as low as 3200. The one rabbit in the tables of Povitzky and Worth treated with killed vaccine (heated) shows a titre of 1600 after the sixth inoculation and no agglutination after the fourth. They also say,

Two rabbits, more responsive to vaccines, developed, after a few inoculations, a comparatively high agglutination titre—up to 1000.

6. Sera from rabbits treated with *Bact. pertussis* grown on blood media developed marked agglutinins for *Bact. pertussis* grown on plain agar.

This is contrary to Bordet's statement that animals inoculated with *Bact. pertussis* grown on Bordet-Gengou medium develop agglutinins for *Bact. pertussis* grown on the same media, not for the organism grown on plain agar; and is in accordance with Povitzky's and Worth's conclusion that,

From our experience it would seem that the culture medium influences an agglutinable strain in so far as it affects its growth and best development, not in its production of different kinds of agglutinins.

Also, serum from a rabbit treated with *Bact. pertussis* No. 0363 (Bordet) grown on *ascitic agar* for the past three years agglutinates all thirteen strains grown on *blood media* (with only one generation, for suspensions, on plain agar); and sera from rabbits treated with thirteen strains grown on blood media agglutinate the *ascitic agar* strains.

7. When *B. bronchisepticus* antiserum is absorbed with *B. bronchisepticus* sufficiently to remove the agglutinins for *B. bronchisepticus*, the agglutinins for *Bact. pertussis* are still unaffected. These unaffected or fixed agglutinins have been called by the writers, *transitive agglutinins*.

Upon repeated absorption, agglutinins for *Bact. pertussis* have been removed by the dog strain of *B. bronchisepticus*, slightly reduced by the monkey strain and affected not at all by the human strain.

It may be that, by the absorption tests, grades of differences are brought out between *B. bronchisepticus* from dog, monkey

and human which are not shown in the agglutination tests. A similar differentiation is thought to have been brought out by complement fixation tests. (See article in press by Ferry and Klíx.)

8. When *B. bronchisepticus* antiserum is absorbed with *Bact. pertussis*, agglutinins for *Bact. pertussis* are removed, but agglutinins for *B. bronchisepticus* are unaffected.

9. When *Bact. pertussis* antiserum is absorbed with *Bact. pertussis*, agglutinins for that organism are removed.

10. When *Bact. pertussis* antiserum is absorbed with *B. bronchisepticus*, agglutinins for *Bact. pertussis* are unaffected.

11. The similar morphology, the identical cultural reactions on differential media, the presence of *Bact. pertussis* agglutinins in artificially produced antibronchisepticus serum and in serum from dogs and rabbits suffering or recovered from distemper, all point toward a close relationship between *Bact. pertussis*, the cause of whooping cough, and *B. bronchisepticus*, the cause of distemper.

REFERENCES.

- BORDET, J. AND SLEESWYK. 1910. Serodiagnostic et variabilité des microbes suivant le milieu de culture. *Ann. d. l'Inst. Pasteur*, 24, 476.
- FERRY, N. S. AND KLÍX, H. 1917. Studies relative to the apparent close relationship between *Bact. pertussis* and *B. bronchisepticus*. II. Complement fixation tests (in press).
- MALLORY, F. B. AND HORNOR, A. A. 1912. Pertussis: The histological lesion in the respiratory tract. *J. Med. Research* 27, 115.
- MALLORY, HORNOR AND HENDERSON. 1913. The relation of the Bordet-Gengou bacillus to the lesions of pertussis. *J. Med. Research* 27, 391.
- MALLORY, F. B. 1913. The pathological lesion of whooping cough. *Boston M. & S. J.* 169, 575.
- POVITZKY, OLGA R. AND WORTH, E. 1916. Agglutination in pertussis. *Archives Int. Med.* 17, 279.

**Studies from the Research Laboratory.
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**A METHOD FOR DETECTING SMALL QUANTITIES OF
CHLORETONE (TRICHLOROTERTIARYBUTYL
ALCOHOL) IN AQUEOUS SOLUTIONS.**

BY T. B. ALDRICH.

(From the Research Laboratory of Parke, Davis & Co., Detroit.)

The wide use of chloretone therapeutically, especially at the present time, as a hypnotic, an anesthetic, a sedative, etc., seems to call for the publication of the following method for its detection.

Part of the work in connection with the development of this method was carried out several years ago, while quite recently this older work has been repeated and the observations made at that time reconfirmed. The efficacy of the method under varying conditions, also its limitations to a certain extent, have been studied.

The method is based on the volatility of chloretone, one of its chief physical properties, its insolubility in water, and also on its property of crystallizing in needles from aqueous solutions on cooling. All who are familiar with chloretone have been impressed with its volatility in the air, or with steam and water. The substance cannot be dried without losing in a very short time considerably in weight; nor can it be kept in an open vessel or even wrapped in ordinary paper any length of time. If kept in a partly filled bottle or container, the compound will be found partly sublimed on the upper walls of the vessel in the form of beautiful needle-shaped crystals.

Although only qualitative results are claimed for the method, still by using duplicate apparatus, especially where small amounts (1 to 5 mg.) are present, the amount may be estimated quite closely. The entire apparatus necessary consists of two flasks, holding 1 liter and 300 cc., respectively, a condenser, and a steam generator. The larger flask is used for the steam distillation, while the smaller is used for the return flow condenser. With

this apparatus, one can detect very readily in 300 to 500 cc. of water 1 to 5 mg. of chloretone, and with a constricted tube cooled with air instead of a condenser, as little as 0.5 mg. or less. In my earlier experiments a constricted air-cooled tube was employed, while later I employed a condenser exclusively.

When a large amount of chloretone is present, it may be observed in the condenser during the steam distillation; but where only a few mg. or a fraction of a mg. are present, the use of the return flow condenser or tube is absolutely necessary for its recognition. The crystals of chloretone in the latter case are noticed usually at the lower cooled portion of the condenser, especially where the steam crest is being condensed, or possibly a few crystals may be observed, according to quantity present, about one-third or at the most half way up the condenser. Where a liquid is employed, it is only necessary to pass steam through it; when a solid contains chloretone this should be powdered and then introduced into the steam distillation flask with the water.

Not only may chloretone be detected with certainty in aqueous solution when alone, but it may also be recognized when mixed with other bodies with which it may be confused, or which may **interfere to a certain extent with its detection.**

In the presence of volatile organic solvents, as alcohol, acetone, etc., it is practically impossible to detect small amounts of chloretone, since the solvent keeps the drug in solution and prevents crystallization. It may be detected with a considerable degree of certainty, however, in a mixture of oil, protein, fat, salt, etc., which does not contain the volatile solvents mentioned above or other volatile bodies with which it may be confused.

Chloretone is not apt to be confused with other volatile compounds of similar use, but, if present to any great extent, may be identified and distinguished from these bodies by further specific tests. The analogous body, brometone, although volatilizing, etc., similar to chloretone, forms, when distilled in small amounts, small crystals instead of needles as in the case of chloretone. Camphor, which also volatilizes with steam, does not give needles as in the case of chloretone, nor does it give the flame test with copper oxide.

Chloral hydrate and chloretone may be confused with each

other, but here the greater solubility of chloral hydrate and the comparative insolubility of chloretone in water, together with its odor, will serve to differentiate these bodies.

In the presence of coagulable protein, I have been unable to detect very small amounts of chloretone, but after digesting the protein with pepsin HCl (thus preventing coagulation) as small amounts may be detected as in water alone.*

Oils or fats do not in general interfere with the detection of small amounts of chloretone.

EXPERIMENTAL.

A. Detection of Chloretone in Aqueous Solution.—5 gm. of chloretone were dissolved in 1,000 cc. of H₂O, and portions of this solution used for the following tests:

(1) To 1 cc. of the chloretone solution (5 mg.), placed in a steam distillation flask, were added about 300 cc. of H₂O. It was distilled with steam until 100 cc. of the distillate had been collected.

This 100 cc. of distillate containing the 5 mg. of chloretone was transferred to a small flask and boiled for half an hour, using a return flow condenser. Crystals of chloretone were noticed in considerable quantity in the condenser. From the amount present in this experiment, it was estimated that as little as 0.25 mg. or even less might be detected.

(2) The above was repeated but with only 0.5 cc. of the chloretone solution (2.5 mg.). *Positive.*

(3) Repeated (2). *Positive.*

(4) Repeated the experiment, using 0.25 cc. of the chloretone solution (1.25 mg.). *Positive, but slight amount.*

(5) Repeated (4). *Positive.*

(6) Repeated the experiment, using 0.05 cc. of the chloretone solution (0.25 mg.). *Negative.*

B. Detection of Chloretone When Dissolved in Horse Serum.—(1) 1 cc. of chloretone solution, 0.5 per cent (5 mg.), was added to 250 cc. of normal horse serum and the test carried out in the usual way. *Negative.* No doubt the coagulation of the serum interfered with the distillation of the chloretone, especially in this small amount.

(2, a) 120 cc. of serum containing 5 mg. of chloretone were digested at 37–39°C. with pepsin hydrochloric acid for 4 days. *Positive. Very pronounced.*

(b) (a) was repeated except that only 1 cc. of chloretone solution (1 mg.) was employed. *Positive. Slight trace.*

C. Detection of Chloretone in the Presence of Water and Oil.—(1) 2 cc. of chloretone solution (10 mg.) were placed in a flask, 200 cc. of distilled water and 0.5 cc. of olive oil added and distilled in the usual way. *Positive.*

*In a paper to be published shortly this feature of digestion is used in detecting chloretone in animal fluids and organs.

(2) 1 gm. of chloretone dissolved in olive oil (5 cc.) was placed in a flask, 200 cc. of water were added, and distilled. *Positive*.

D. Detection of Chloretone When Mixed with Various Substances.—The following substances were placed in a steam distillation flask:

0.5 cc.	propionic acid.
0.5 "	butyric "
0.2 gm.	stearic "
0.2 "	palmitic "
1.0 "	dextrose.
0.5 "	dried horse serum

To the above mixture 1 cc. of chloretone solution 0.5 per cent was added (5 mg.) and then 150 cc. of water. This mixture was subjected to steam distillation. Chloretone *positively recognized*.

E. Detection of Chloretone in Alcoholic Solution of Various Strengths.—(1) 1 cc. of an aqueous 0.5 per cent solution of chloretone (5 mg.) was placed in a flask, 100 cc. of 10 per cent alcohol were added, and distilled with steam. *Negative*.

(2) Repeated (1) except that 100 cc. of 5 per cent alcohol were used. *Negative*.

(3) Repeated (1) except that 100 cc. of 1 per cent alcohol were employed. *Negative*.

From experiments carried out with organic solvents (alcohol) it is shown that chloretone, especially in small amount, 1 to 5 mg., cannot be detected by this method even in the presence of a very small amount of alcohol. As far as investigated, even small amounts of volatile organic solvents dissolve the small amount of chloretone present, thus preventing its recognition.

F. Detection of Chloretone When Mixed in Small Quantities with Other Volatile Compounds. The following products were placed in a steam distillation flask, about 300 cc. of water added and distilled until about 100 cc. of distillate had passed over.

(1) 5 mg. brometone crystals.

(2) 1 cc. chloretone inhalant.

(3) 1 gm. camphor.

(4) 15 mg. camphor, 15 mg. chloretone.

(5) 0.2 gm. chloral hydrate.

(6) 15 mg. chloral hydrate, 15 mg. of chloretone.

(1) Did not give typical needles as by chloretone.

(2) Semicrystalline sublimation obtained. Chloretone may be detected in this product by means of the copper-flame test (green).

(3) Did not give typical needles (applied copper-flame test). *Negative*

(4) Did not give typical needles (applied copper flame test). *Positive*

(5) Did not give typical crystals; solubility of portion sublimed in water distinguished from chloretone.

(6) Did not give very pronounced needles; however, some were present. Was separated by water.

The method lends itself especially to the detection of chloretone in toxicological and pharmacological work. Here we have the drug usually unassociated with bodies that would interfere with its recognition, and a very small amount may be detected.

SUMMARY.

Very small quantities of chloretone may be detected with certainty in aqueous solutions by distilling with steam and boiling the distillate on a reflux condenser.

The method employed is based on the physical properties of chloretone: volatility with steam, insolubility in water, property of crystallizing in the form of needles.

Chloretone may be detected when accompanied by other substances such as oils, fats, acids, salts, etc. The organic solvents, however, prevent its recognition.

In the presence of substances which simulate chloretone, specific tests may be made to differentiate them.

The method lends itself admirably to the detection of the drug in the fluids and tissues of the body.

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**A BOTHRIOCEPHALID TAPEWORM FROM THE DOG
IN NORTH AMERICA, WITH NOTES ON
CESTODE PARASITES OF DOGS.**

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Although at least four species of bothriocephalid tapeworms have been reported from the dog in various parts of the world, there were no records of the sort from the United States until recently. The first record of which we are aware is one by Van Es and Schalk (1917), who report what they call *Dibothriocephalus latus* from a dog at Agricultural College, N. D. Their specific determination is apparently casual, being only incidental to work on anaphylaxis, and is presumably based on the fact that the worm was a bothriocephalid tapeworm and that *D. latus* is one of the commonest and best-known of these worms from the dog. We wish to report another case of the occurrence of a bothriocephalid tapeworm in the dog in this country.

Four specimens of a species of bothriocephalid worm were collected by us from the small intestine of an experiment dog, No. 140, at Detroit on July 27, 1917. These specimens are all small, measuring respectively 7.5, 14.5, 16 and 36 mm. in length and very narrow, the largest specimen being less than 2 mm. wide. The head of the largest specimen is 1.64 mm. long; that of the next largest is 1.5 mm. long; that of the next largest is 1.37 mm. long; that of the smallest is 1.66 mm. long. The width of the head is about 0.4 mm. in the narrow transverse diameter across the bothridial aperture and about 0.7 mm. in the dorsoventral diameter from the external margin of one bothridium to the external margin of the other. There is no neck, the head arising directly from the anterior margin of the first segment. In stained toto mounts, the primordia of the genitalia appear 3 to 3.33 mm.

posterior of the head (Fig. 1). They first appear as diffusely spherical objects, later becoming dumbbell-shaped, the dumb-

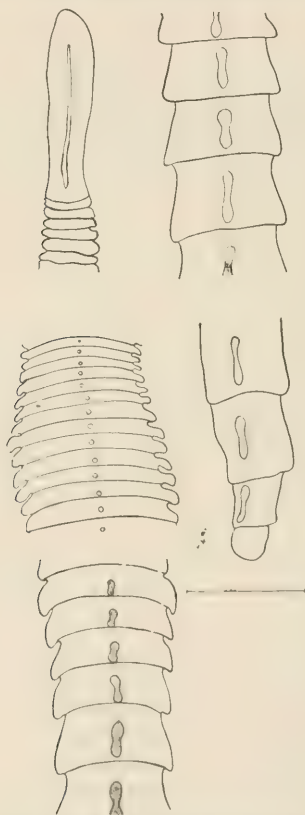


FIGURE 1. *Diphyllbothrium americanum*.
Camera lucida sketch of portions of strobila.

bell subsequently elongating. There are no eggs present in any of the strobila and the genitalia appear to be immature; it is

possible that they are abortive and sterile. Malformations and displacements of the genitalia are common. The last normal segment of the largest worm, about 7 mm. from the posterior end, is 558 μ long and 1.34 mm. wide. In the next largest worm, the largest segment, not far from the posterior end, is 668 μ long and 833 μ wide.

The bothriocephalid cestodes of the dog were at one time referred to the genus *Bothriocephalus* Rudolphi, 1908, of which the type is *B. punctatus* (Rudolphi, 1802) Rudolphi, 1810, from various fish. They were later referred to the genus *Dibothriocephalus* Luehe, 1899, of which the type is *D. latus* (Linnæus, 178) Luehe, 1899, but in 1910 Luehe regards his genus *Dibothriocephalus* as a synonym of *Diphyllobothrium* Cobbold, 1858, of which the type is *D. stemmacephalum* from *Delphinus phocaena*, and the bothriocephalid worms from the dog are now generally referred to *Diphyllobothrium*. These worms are briefly described as follows:

D. latum attains a length of 2 to 9 meters. It has a head 2 to 3 mm. long according to Braun (1906) and Fiebiger (1912) and 2 to 5 mm. long by 0.7 to 1 mm. wide according to Neveau-Lemaire (1912). These writers agree that the neck is thin, its length depending on the state of contraction. It would appear from the fact that our strobilae are only a few centimeters long, instead of several meters, and that the heads are only 1.37 to 1.66 mm. long and 0.4 to 0.7 mm. wide, and from the fact that they have no neck, that we have a form other than *D. latum*, and we believe that such is evidently the case.

In this connection it is interesting to note that Nickerson (1906) notes a case of *D. latum* in a child born in Minnesota and who had never been out of that State, the first record of an infection with this worm where the infection is known to have been acquired in this country. Nickerson says the head of the worm was 1.75 mm. long and 0.9 mm. wide. These figures are intermediate between those given for *D. latum* and those for our specimens. Nickerson states, regarding *D. latum*: "There are no reports of its being found in American dogs, cats or foxes—the other animals which are known to serve as definitive hosts for the parasite." He also states: "Larvae of *Dibothriocephalus* do occur in

American fishes. I have obtained them from fish caught in the Great Lakes, but without feeding experiments to rear the adult worm from the larva it is impossible to determine the species of *Dibothriocephalus* and the probability is in favor of such larvae being of some species other than *latus*—the parasite of man."

Diphyllbothrium cordatum, another parasite of dogs, has a characteristic heart-shaped head, 2 mm. by 2 mm. in diameter, and it is certain that the species we have found is not *D. cordatum*.

D. fuscum was described from the dog in Iceland by Krabbe. According to Neveau-Lemaire (1912), this worm has a compressed lanceolate head, a neck slightly narrower than the head, followed by segments which are at first indistinct. In our specimens there is no neck and the first segments, though small, are quite distinct. It is, however, difficult to make distinctions of this sort. We are frankly unable to ascertain with any certainty whether our specimens correspond to the meager description of *D. fuscum*, and we merely assume that since the agreement is not exact, it is fairly unlikely that rare material from such widely separated localities should be the same species.

D. serratum is named as a parasite of dogs by a number of writers, but is then disregarded and no description is available to us. Some writers list it with a question mark, indicating uncertainties in regard to it, and we are compelled to disregard it.

Bothriocephalus spiratus is listed by Neveau-Lemaire (1912) as found in the dog in Italy. Such a species is not listed by Geddoelst (1911) or Stiles and Hassall (1912), and it seems likely that this is a printer's error for *B. serratus*, which is not named in Neveau-Lemaire's list of dog parasites.

In order that the species found here by us may have some name to which it may be referred, we propose for it the name of *Diphyllbothrium americanum*. Should it develop later that this name is a synonym of some existing name, it will be easy to suppress the synonym. In the meantime, we believe it is more convenient to have a name and we are following the advice of Stiles in such matters—that it is better to give a new name which may be later suppressed than to confuse two species under one name. Of course, we do this in the belief that this species cannot be

identified with *D. latum*, *D. cordatum* or *D. fuscum* and it is not feasible to make a comparison with *D. serratum*. Specimens will be deposited in the collection of the U. S. Bureau of Animal Industry, where they will be available for future examination.

It might be noted here that the bothriocephalid larvae, or plerocercoids, found by Nickerson in fish from the Great Lakes may have been the larvae of this species, an idea which is in agreement with Nickerson's statements. The idea is of interest, as bothriocephalids parasitic in man are commonly capable of parasitizing dogs, and vice versa. It may be, therefore, that fish caught in the Great Lakes and consumed here in Detroit and elsewhere are parasitized by a plerocercoid other than that of *D. latum* but possibly capable, nevertheless, of parasitizing man.

The following key to the bothriocephalid worms of the dog makes use of such distinctions as can be drawn in view of the scarcity of detailed description:

1. No available description.....*Diphyllbothrium serratum*
 Descriptions available2
2. Heart-shaped head, 2 mm. by 2 mm. in diameter.....*D. cordatum*
 Head not heart-shaped, longer than wide.....3
3. No neck, the first segment following immediately behind head....
 *D. americanum*
 Neck present4
4. Neck slightly narrower than the head.....*D. fuscum*
 Neck thin; strobila attains a length of 2 to 9 meters.....*D. latum*

It is possible, even probable, that *D. americanum* is normally a parasite of wild carnivores.

In this connection, it might be noted that the life history of the bothriocephalid tapeworms has just been ascertained by Janicki and Rosen (1917; 1918). It has long been known that the larval form occurred in fish and that the adult worm developed in suitable hosts when raw fish were ingested, but attempts to infect fish with the eggs or embryos of the adult tapeworm have always been unsuccessful. Janicki and Rosen met with no better success in feeding experiments of this sort than other investigators had achieved, so they began a search for an intermediate host capable of becoming infested by the embryos of the worm and in turn infesting the fish. In this search they were successful, the intermediate hosts found being small inver-

tebrate animals known as copepods. Of these, *Cyclops strenuus* and *Diaptomus gracilis* were found to function as hosts. The ciliated embryo of the worm was found to be taken into the digestive tract of the copepod; from there it penetrated the wall of the intestine and transformed in the body cavity into an intermediate larval form, the proceroid, which is armed with hooks on a globular caudal appendix. Not more than two of these were found in one host. When these infested copepods were eaten by fish, they were digested and the larval worms set free. The proceroid loses its hooks and the caudal appendix, if these have not already been lost in the copepod, and the plerocercoid thus formed traverses the wall of the stomach, attains the body cavity and thence enters the musculature or the liver. The parasitized copepods lose their active movements and move slowly along at the bottom of the water. This admirable piece of work by Janicki and Rosen furnishes us with the first case of a tapeworm having two intermediate hosts with two larval stages and will doubtless open a large field of investigation and theorizing.

We take this occasion to summarize briefly the status of the dog tapeworms as regards their occurrence on this continent, so far as records are available to us. Most of the common species of dog cestodes have been reported from the dog on this continent, and some rare forms have been found. Other tapeworms, some of which have only been reported as found once in the dog in Europe, are not known on this continent.

Mesocestoides lineatus has been reported by Stiles and Hassall (1894) as represented in Leidy's collection by specimens collected from an Esquimaux dog by Kane.

Dipylidium caninum is a common parasite of dogs in the United States. It has been reported from children at least three times, in cases in Detroit, Mich., Ithaca, N. Y., and Norwich, Conn.

Dipylidium sexcoronatum has been reported from dogs in the United States at Bethesda, Md., and Detroit, Mich., by Hall (1917). We find it fairly often here at Detroit and our impression is that it is as common here as *D. caninum*. The strobila is much narrower than *D. caninum*. Some of the specimens with a narrow strobila appear to have only 5 rows of hooks and

should be studied with a view to determining whether *D. sex-coronatum* has sometimes 5 rows of hooks, as well as 6 rows, or whether this material belongs to a new species.

Of 200 dogs examined by us at Detroit, 46 per cent had *Dipylidium*, but other investigations did not permit of taking time to determine the species in all these cases. The average number of worms present in a dog was 14.8; the largest number was 205 and the next largest 100.

Taenia pisiformis (*Taenia serrata*) is probably the commonest of the dog tapeworms belonging to the genus *Taenia*. Ward (1897) found it present in 45 per cent of the dogs examined by him at Lincoln, Neb. As long as dogs have access to our wild hares and rabbits, the hosts of the larval tapeworm, this worm will probably be common in dogs. It is naturally commoner in dogs in the country and in villages and small cities, and less common in large cities, where the dogs seldom get outside the city and where the rabbits and other game are killed off for some distance out from the city. At Detroit we found this parasite in 6 dogs, 3 per cent of our 200 dogs, the largest number present being 7 and the next largest 5. A dog not in this series of 200 had 20 *T. pisiformis*.

Taenia hydatigena (*Taenia marginata*) is still fairly common in dogs in the United States, but it appears to be of less common occurrence than was the case 10 or 20 years ago. The increased application of adequate meat inspection to the abattoirs of the United States and the increased care in the disposal of slaughter-house refuse will make this worm increasingly scarcer and it will eventually, and perhaps very soon, disappear. We only found it in 2 dogs, 1 per cent, of our 200 dogs here, and suspect that these dogs probably acquired the infection in the country, where the primitive methods of disposing of viscera of slaughtered animals by feeding to the dogs still prevail in some sections. As more and more farmers learn the impropriety and danger of such practices, it is likely that this will be one of the first dog tapeworms to become extinct.

Taenia krabbei was reported from Alaska by Ransom (1915), the adult tapeworm being obtained by feeding the corresponding bladderworm from the reindeer to dogs. Ransom (1913) had

previously reported the larva of this tapeworm from reindeer in Alaska.

Taenia ovis has been experimentally developed in dogs in this country by Ransom (1913), by feeding larvae collected from the muscles of sheep. Ransom reports the larvae from sheep in Montana, Idaho, Washington, Oregon, California, Colorado and Nevada.

Taenia balaniceps was described from the dog in Nevada and the lynx in New Mexico by Hall (1910).

Multiceps multiceps, the gid tapeworm, has been experimentally developed in dogs in this country by Hall (1909) and by Taylor and Boynton (1910). The larva, or gid bladderworm from the brain, has been reported from sheep in this country a number of times and has been enzootic for many years in northern Montana. Ransom (1913) reports gid as present in Arizona and occasioning the loss of a considerable number of sheep.

Multiceps serialis has been experimentally developed in the dog in this country by Hall (1910) and has been reported from dogs in natural infestations by Ward (1897), Stevenson (1904) and Ransom (1905). The bladderworm, or larval stage, is common in jack-rabbits in many parts of the western United States, occurring in the subcutaneous and connective tissues.

Echinococcus granulosus (*Taenia echinococcus*) was reported from the dog in this country by Stiles and Hassall (1894), the specimens being collected by Curtice at Washington, D. C. This tapeworm has also been reported from the dog in Alaska by Ransom (1915).

REFERENCES.

- BRAUN, MAX. 1906. The Animal Parasites of Man. London. 453 pp., 290 figs.
- IEBIGER, JOSEF. 1912. Die tierischen Parasiten der Haus- und Nutztiere. Wien u. Leipzig. 424 pp., 303 figs.
- GEDOELST, L. 1911. Synopsis de Parasitologie de l' Homme et des Animaux domestiques. Liège et Bruxelles. 332 pp.
- HALL, MAURICE C. 1909. A discussion of de Renzi's treatment of somatic taeniasis with male fern, and some tests of the treatment in gid. *Amer. Vet. Rev.*, Vol. 36 (3), Dec., pp. 328-337.
1910. The gid parasite and allied species of the cestode genus *Multiceps*. I. Historical Review. *U. S. Bu. Anim. Indust. Bull.*, 125, pt. 1, 68 pp.
1910. A new species of cestode parasite (*Taenia balaniceps*) of the dog and of the lynx, with a note on *Proteocephalus punicus*. *Proc. U. S. Nat. Mus.* (1870), Vol. 39, pp. 139-151, 9 figs.
1917. Parasites of the dog in Michigan. *Jour. Amer. Vet. Med. Ass'n*, N. S., Vol. 4 (3), June, pp. 383-396.
- JANICKI, C., AND ROSEN, F. 1917. Le cycle évolutif du *Dibothriocephalus latus*. Recherches expérimentales et observations. *Bull. Soc. Neuchâtel des Sci. Nat.*, Vol. 42, pp. 19-53. Not seen.
1917. Idem. *Bull. Inst. Pasteur, Rev. et anal.*, Vol. 15 (24), 30 Déc., pp. 769-770. Rev. by Roubaud.
- NEVEAU-LEMAIRE, MAURICE. 1912. Parasitologie des Animaux domestiques. Maladies parasitaires non bactériennes. Paris, 1257 pp., 770 figs.
- NICKERSON, W. S. 1906. The broad tapeworm in Minnesota, with the report of a case of infection acquired in the State. *Jour. Amer. Med. Ass'n*, Vol. 46 (10), March 10, pp. 711-713.
- RANSOM, BRAYTON H. 1905. The gid parasite (*Coenurus cerebralis*): Its presence in American sheep. *U. S. Bu. Anim. Indust. Bull.* 66, 23 pp., 12 figs.
1913. *Cysticercus ovis*, the cause of tapeworm cysts in mutton. *Jour. Agri. Research*. Vol. 1 (1), Oct. 10, pp. 15-58, pls. 2-4, 13 text figs.
1913. The Zoological Division. In *Rep. of Chief of Bu. of Anim. Indust.* for the year ending June 30, 1913; pp. 31-33.
1915. The Zoological Division. In *Rep. of Chief of Bu. of Anim. Indust.* for year ending June 30, 1915; pp. 58-60.
- STEVENSON, EARLE C. 1904. Variations in the hooks of the dog tapeworms, *Taenia serrata* and *Taenia serialis*. *Studies Zool. Lab.*, Univ. of Neb., (59), pp. 409-429, 6 pls.
- STILES, CH. WARDELL, AND HASSALL, ALBERT. 1894. A preliminary catalogue of the parasites contained in the collections of the United States Bureau of Animal Industry, United States Army Medical Museum, Biologic Department of the University of Pennsylvania (Coll. Leidy) and in Coll. Stiles and Coll. Hassall. *Vet. Mag.*, Vol. 1 (4), Apr., pp. 245-253; (5), May, pp. 331-354.
1912. Index Catalogue of Medical and Veterinary Zoology. Subjects: Cestoda and Cestodaria. *Hyg. Lab. Bull.* 85, 467 pp.
- TAYLOR, WALTER J., AND BOYNTON, WM. H. 1910. Gid found in sheep in New York. *Ann. Rep. N. Y. State Vet. Coll. for 1908-09*, pp. 69-77, 3 pls.
- VAN ES, L., AND SCHALK, A. F. 1917. Notes on parasitic anaphylaxis and allergy. *N. D. Agri. Exp. Sta. Bull.* 125, pp. 151-193, fig. 1.
- WARD, HENRY B. 1897. Animal parasites of Nebraska. *Rcp. Neb. State Bd. Agri. for 1896*, pp. 173-189, 12 figs.

Studies from the Research Laboratory.

Parke, Davis & Co.

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NOTES ON THE ACANTHOCEPHALID AND ARTHROPOD PARASITES OF THE DOG IN NORTH AMERICA.

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This paper is primarily intended to cover additional information regarding the rare thorny-headed worm of the dog, but in view of the fact that the writers are summarizing in other papers, published (Hall and Wigdor, 1918) and in manuscript, the available data as to the occurrence of protozoan, cestode, trematode and nematode parasites of the dog in North America, in connection with some new findings, a summary of our knowledge of arthropod parasites is included in this paper in order to complete a series covering the parasites of dogs in North America. These summaries are intended to be comprehensive, but not exhaustive. Not all records of a given parasite are cited and by no means all the literature possibly involved has been examined.

ACANTHOCEPHALA. *Echinorhynchus canis* was described from this country by Kaupp (1909) on 4 specimens collected from a dog at San Antonio, Texas. No subsequent writers have reported this species. Dr. Clifford C. Whitney collected one specimen of what is evidently this species from a young, black and white, male mongrel dog at College Station, Texas, on March 8, 1917, and sent it to one of us (Hall) at Detroit. It appears, therefore, that this rather rare parasite is established in Texas, at least.

Through the courtesy of Dr. Whitney, we were allowed to retain his specimen for study. A brief description is as follows:

The worm is a female, 14 mm. long. The proboscis is armed with 6 rows of hooks, the largest set anteriorly. The hooks in the 4 anterior rows have anterior and posterior roots resembling the handle and guard of the taenioid cestode hook; those in the 2

posterior rows are rosethorn-shaped (see figs.). The proboscis shows 33 hooks, of which 18 are large and 1 small. It is likely that 3 of the small hooks have been lost and that the total number present should be 36. The proboscis is 2 mm. long. The mounted specimen shows that the body is distinctly annulated. The body form and outline is substantially that figured by Kaupp (1909). There are eggs present, but apparently these have not been fertilized (this was the only specimen present in the host animal) and segmentation has not begun.

The cuticular annulation, the proboscis structure and the hooks indicate that this worm belongs in the family Gigantorhynchidae. As regards its generic position, it is difficult to ascertain this accurately by an examination of the one immature femal speci-

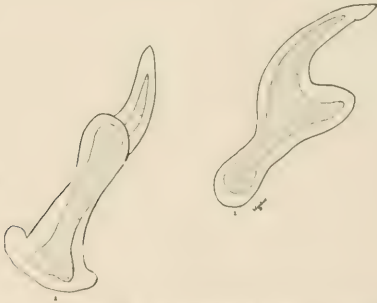


FIG. 1. *Gigantorhynchus canis*.
Large hooks from first and second rows as numbered $\times 180$.

men in our possession, but it appears to have the essential characters and appearance of the genus *Oncicola*, Travassos, 1916, and it is accordingly designated *Oncicola canis* (Kaupp, 1909) Hall and Wigdor, 1918.

In view of the fact that Acanthocephala forms have been reported from the dog in Europe, the question naturally comes up as to whether the European and American forms are identical. The European species is listed by Railliet (1893), Neveau-Lemaire (1912) and other recent writers as *Echinorhynchus grassei* Deffke, 1891. An examination of Deffke's (1891) paper,

shows that he has in his list of dog parasites "*Echinorhynchus grassii*, 1888." This is evidently not an attempt to name an *Echinorhynchus* species after Grassi, as the date after Grassi's name shows, but is a reference to Grassi's paper of that date regarding this parasite. So far as we can ascertain, Grassi and Calandruccio (1888) listed an *Echinorhynchus* from the dog in Sicily; they state that Sicily is an exception to the rule that in general *Echinorhynchus* is rare in mammals other than swine, and that not infrequently they found an *Echinorhynchus*, probably a new species, in the small intestine of the dog. Travassos (1917) makes *Echinorhynchus grassii* a synonym of *Moniliformis*, which eliminates the possibility that the European and American forms are identical. Granting the accuracy of the Brazilian authority's synonymy, it follows that the dog is parasitized at times by at least 2 echinorhynchs, *Moniliformis moniliformis* and *Oncicola canis*. The latter is probably a customary parasite of some Texan carnivore other than the dog. *O. canis* is very similar to *O. onicola* (v. Ihering, 1892). Both species show asymmetrical hook bases in some hooks and recurved projections on hook tips, but the hook measurements are so dissimilar as to make it quite unlikely that the species are identical.



FIG. 2. *Gigantorhynchus canis*.
Small hooks from fifth and sixth rows as numbered x240.

Travassos gives measurements for 4 types of hooks in *O. onicola*, the first type being the hooks of the first and second (or anterior) rows, the second type the hooks of the third row, the third type the hooks of the fourth row, and the fourth type the hooks of the fifth and sixth (or posterior) rows. His measurements and ours are as follows:

Species.	Hook type.	Distance, tip of blade to tip of apical root (microns).	Distance between root extremities (microns).
<i>O. onicola</i>	First.....	348	177
<i>O. canis</i>	First.....	200	148 to 160
<i>O. onicola</i>	Second.....	268	149
<i>O. canis</i>	Second.....	140	50
<i>O. onicola</i>	Third.....	227	130
<i>O. canis</i>	Third.....	166	116
<i>O. onicola</i>	Fourth.....	120	—
<i>O. canis</i>	Fourth.....	130	—

In the measurements of hooks of the third type, we have measured from the tip of the asymmetrical process on the root, as Travassos appears to have done. Without this projection, the distance from the tip of the blade to the tip of the apical root is $140\ \mu$; that between the root extremities is 60 to $64\ \mu$. *O. onicola* occurs in *Felis* (*Leopardus*) *onca* and *Felis* (*Catopuma*) *jaguarundi*, and it is quite likely that *O. canis* is normally parasitic in some of the Felidae.

ARTHROPODA. *Otobius megnini* (*Ornithodoros megnini*) has been reported from dogs in the southern United States by Hooker, Bishopp and Wood (1912).

Ixodes ricinus is reported from dogs in Canada by Hewitt (1915).

Ixodes scapularis is reported from dogs in the southern United States by Hooker, Bishopp and Wood (1912).

Ixodes cooki is reported from dogs in Canada by Hewitt (1915) and in the United States by Banks (1908).

Ixodes kingi is reported from dogs in the western United States by Hooker, Bishopp and Wood (1912).

Ixodes pratti is reported from the dog in Canada by Hadwen according to Hewitt (1915).

Rhipicephalus sanguineus, the brown dog tick, has been collected from the dog in Texas and in Mexico, according to Hooker, Bishopp and Wood (1912). Specimens of this tick from a dog on one of our battleships have been sent to this laboratory for identification, with the statement that the dog apparently became infested in New Orleans.

Margaropus annulatus, the cattle tick, has been reported from dogs in the southern United States by Hooker, Bishopp and Wood (1912) and other writers, but it is very rare on this host.

Amblyomma maculatum has been reported from dogs in Texas and Louisiana by Hooker, Bishopp and Wood (1912).

Amblyomma americanum has been reported from dogs in Texas by Hooker, Bishopp and Wood (1912).

Amblyomma cajennense has been reported from the dog at Panama by Hooker, Bishopp and Wood (1912).

Dermacentor andersoni (*D. venustus*) has been reported from dogs in the western United States by Hooker, Bishopp and Wood (1912), by Stiles (1910) and by others.

Dermacentor occidentalis is reported from the dog in the Pacific coast region of the United States by Hooker, Bishopp and Wood (1912).

Dermacentor variabilis is a common and widely distributed parasite in the United States, the dog being the usual host, and has been reported from the dog in Canada by Hadwen (1912).

Sarcoptes scabiei canis is the cause of sarcoptic mange in the dog. It appears to be much less common in the United States than demodectic mange.

Demodex folliculorum canis, the cause of demodectic mange in dogs, is common in the United States. We have had a number of cases here at Detroit.



FIG. 3. *Gigantorhynchus canis*.
Hooks from different rows as numbered x75.

Linognathus piliferus, the sucking louse of the dog, appears to be more common on the west coast of the United States than in the East. We have found a few cases here at Detroit.

Trichodectes latus, the biting louse of the dog, is fairly common in the United States. In connection with tests of the efficacy of sodium fluoride against biting lice, proposed by Bishopp and Wood (1917) for use against biting lice of poultry and reported by Hall (1917) as effective against biting lice of the horse, we have made some tests in this laboratory of its efficacy against the biting louse of the dog and find it effective. We have seen no bad results from it, but the possibility of trouble from poisons ingested through licking the hair and skin must be kept in mind in treating dogs. In a test to determine the toxicity of sodium fluoride, we gave a 9-kilo dog 1 gram of sodium fluoride in a gelatine capsule, followed by a small amount of water. The dog seemed in fairly good health for 3 days, but was found dead on the fourth day; there was a severe gastro-enteritis, with some hemorrhage, and an acute nephritis.

Ctenocephalus canis, the dog flea, is common on dogs in the United States.

Pulex irritans, the human flea, is not an uncommon parasite of dogs, especially on the west coast of the United States.

Echidnophaga gallinacea, the stick-tight flea, is often found on the ears of dogs in the southern and southwestern part of the United States, according to Bishopp (1915).

Gastrophilus intestinalis (*G. equi*), *G. nasalis* and *G. hemorrhoidalis* have been reported from the dog in experimental infestations in the United States by Hall (1917). *G. intestinalis* functions readily as an incidental parasite of the dog; *G. nasalis* did not adapt itself to the dog so readily; *G. hemorrhoidalis* apparently had little or no capacity for parasitism in the digestive tract of the dog.

Cochliomyia macellaria (*Chrysomyia macellaria*), the screw-worm, is a common parasite of domesticated animals in the southern United States. Dunn (1918) has recently recorded a case of infestation in the dog at Ancon, Panama.

Cuterebra emasculator, the rabbit bot, has been collected from the scrotum of the dog, apparently in North America, according to Geddoelst (1911).

Dermatobia cyaniventris (*D. hominis*) is reported as parasitic in dogs in tropical America by Verrill, according to Osborn (1896), and others.

Myiasis, due to infestation with various dipterous larvae, is not uncommon among dogs in the United States. We have several times seen cases of rectal myiasis in the dog, especially dogs that were sick and weak, and more particularly those that had diarrhea or blood in the feces. Fish (1910) records an interesting case of cutaneous myiasis, where a collie pup was found to have hundreds of maggots in the dense hairs along the spine from the neck to the scrotum, many of the maggots being embedded in the skin. The species of fly responsible for these infestations is commonly not ascertained.

Simulium molcstum is reported by Packard, according to Osborn (1896), as attacking the Newfoundland dogs of Labrador and driving them to take to the river for protection.

Simulium pecuarum is recorded in reports of the U. S. Department of Agriculture, according to Osborn (1896), as attacking dogs in the United States.

Stomoxys calcitrans is reported by Hewitt (1917) as attacking dogs in Canada.

Tabanus lineola and *T. trijunctus* are reported by Snyder (1917) as annoying dogs in southern Florida.

There are numerous other biting flies that attack dogs on occasions, but the records are frequently indefinite and uncertain.

BIBLIOGRAPHY.

- BAXEN, N. A. 1908. A revision of the Ixodoidea, or ticks, of the United States. *U. S. Bur. Ent. & Pl. Tech. ser.* (15), June 6, 61 pp., 10 pls.
- BISHOPP, F. C. 1917. Fleas and their control. *U. S. Farm Bull.* (897), 15 pp., 5 figs.
- COTE, L. E. N. J., and PHILLIP B. HADLEY. 1910. Blackhead in turkeys: A study in avian coccidiosis. *R. I. Agric. Exp. Sta. Bull.* (141), pp. 137-271, 11 pls.
- DEGEN, O. 1891. Die Entozoen des Hundes. *Arch. f. wissenschaftl. u. prakt. Thierh.*, Berl., v. 17 (1-2), pp. 1-60; (4-5), pp. 253-289, pls. 1-2, figs. 1-11.
- DUNN, L. H. 1918. Studies on the screw worm fly, *Chrysomya macellaria* Fabricius, in Panama. *J. Parasitol.*, v. 4 (3), March, pp. 111-121.
- FISH, P. A. 1910. A fly-blown and distempered dog. *Rept. N. Y. St. Vet. Coll. for yr. 1908-1909*, pp. 48-49.
- GEDDES, L. 1911. Synopses de parasitologie de l'homme et des animaux domestiques. Lierre et Bruxelles. xx+232 pp., 327 figs.
- GRASSI, GIOVANNI BALDINI, and SALVATORE CALANDRANO. 1888. Ueber einen Echinorhynchus, welcher auch in Menschen parasitirt und dessen Zwischenwirth ein Blaps ist. *Centralbl. f. Bakteriologie, Parasitenk.*, Jena, 2. J., v. 3 (17), pp. 521-525, figs. 1-7.
- HALL, MAURICE C. 1917. Parasites of the dog in Michigan. *J. Am. Vet. Med. Ass'n.*, v. 4 (3), June, pp. 385-396.
1917. Notes in regard to horse lice, *Trichodectes* and *Haematopinus*. *J. Am. Vet. Med. Ass'n.*, v. 4 (4), July, pp. 494-504.
1917. Notes in regard to bots, *Gastrophilus* spp. *J. Am. Vet. Med. Ass'n.*, v. 5 (2), Nov., pp. 177-184.
- HALL, MAURICE C., and MARY W. GIER. 1918. Canine coccidiosis, with a note regarding other protozoan parasites from the dog. *J. Am. Vet. Med. Ass'n.*, v. 6 (1), Apr., pp. 64-76, 1 fig.
1918. A bothriocephalid tapeworm from the dog in North America, with notes on cestode parasites of dogs. *J. Am. Vet. Med. Ass'n.*, v. 6 (3), June, pp. 355-362, 1 fig.
- HEWITT, C. GORDON. 1915. A contribution to a knowledge of Canadian ticks. *Trans. Roy. Soc., Canada*, ser. 3, v. 9, sec. 4, pp. 225-239, 3 pls., 1 map.
1917. Report of the Dominion Entomologist, C. Gordon Hewitt, D.Sc., F.R.S.C., for the year ending March 31, 1916. 73 pp., 9 figs.
- HOOVER, W. A., F. C. BISHOPP and H. P. WOOD. 1912. The life history and bionomics of some North American ticks. *U. S. Bur. Entom. Bull.* (106), Sept. 7, 239 pp., 15 pls., 17 text figs.
- KATIE, R. F. 1909. *Canine leishmaniasis*. *Am. Vet. Rev.*, v. 35 (2), May, pp. 154-155, 3 figs.
- NEVEAU-LEMAIRE, MAURICE. 1912. Parasitologie des animaux domestiques. Paris. 1257 pp., 770 figs.
- OSBORN, HERBERT. 1896. Insects affecting domestic animals. An account of the species of importance in North America, with mention of related forms occurring on other animals. *Dir. Entom. Bull.* (5), n. e., 302 pp., 170 figs.
- RAILLIET, A. 1893. Traité de zoologie médicale et agricole. 2 éd. (fasc. 1), 736 pp., 191 figs., Paris.
- SNYDER, T. E. 1917. Notes on horse flies as a pest in southern Florida. *Proc. Entom. Soc., Washington*, v. 18 (4), pp. 208-210. Dated Dec., 1916; actually published June 11, 1917.
- STILES, CH. WARDELL. 1910. The taxonomic value of the microscopic structure of the stigma plates in the tick genus *Dermacentor*. *Hyg. Lab. Bull.* (62), 72 pp., 43 pls.
- TRAVASSOS, LAURO. 1917. Contribuições para o conhecimento da fauna helmintológica brasileira. vi. Revisão dos acantocefalos brasileiros. Pt. I. Fam. Gigantorhynchidae Hirst, 1892. *Mem. e Ann. Acad. Bras. Ci.*, v. 9 (1), 11, 5-62, pls. 1-24A.

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**STUDIES RELATIVE TO THE APPARENT CLOSE
RELATIONSHIP BETWEEN BACT. PERTUSSIS
AND B. BRONCHISEPTICUS.***

II. COMPLEMENT FIXATION TESTS.

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In a previous article (Ferry and Noble, 1918) we have described the cultural, agglutination and absorption reactions between *Bact. pertussis* and *B. bronchisepticus* and have shown that, while the two organisms are distinct, they are apparently somewhat closely related. The most striking characteristics of the organisms, according to the serological reactions, were shown to be the ability of *B. bronchisepticus* to produce an immune serum that would agglutinate both the *B. bronchisepticus* and *Bact. pertussis* antigens and the ability of *Bact. pertussis* to produce an immune serum that would agglutinate only the homologous antigen. The absorption reaction showed that the *B. bronchisepticus* antigen would absorb from the antibronchisepticus serum (a serum that contained agglutinins for both organisms) only the *B. bronchisepticus* agglutinin (the major agglutinin), leaving intact the agglutinin for *Bact. pertussis* (the minor agglutinin). This minor agglutinin could only be absorbed by the *Bact. pertussis* antigen. This type of agglutinin was termed by the authors a "transitive" agglutinin.

The present investigation was undertaken to confirm the work of the previous paper through complement fixation tests and to determine, if possible, the value of this test in differentiating between the two organisms.

Strains used. At first a large number of strains of each organism were used, the same strains as those worked with in the previous experiments already described, but as it was found that

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all strains of the same organism gave similar reactions it was deemed advisable to cut down the number to three of each in order to save time. Of the *B. bronchisepticus*, No. 36 (dog), No. 123 (monkey) and human strains were tested; and of *Bact. pertussis*, No. 0363 (Bordet), No. 109 and No. 248 (Povitzky).

Technic. For the volume of the complement fixation tests it was found more satisfactory to use 2 cc. than 5 cc. as advised for the Wassermann test or 0.5 cc. suggested by Olmstead and Povitzky in a serological comparison of the Bordet-Gengou bacillus and hemoglobinophilic bacilli. The hemolytic system was composed of sheep cells in a 2 per cent suspension, guinea-pig complement in a 1 to 10 dilution and rabbit amboceptor in 1 to 1500 dilution. Complement titration was made by using 0.1 cc. of amboceptor and varying amounts of complement.

In determining the relationship of the various strains two units of both amboceptor and complement were employed. All titrations were incubated one hour before and one hour after the addition of the sensitized cells, at 32° C. The dilution was chosen in which complete hemolysis was produced, readings being made at the end of the hour's incubation. All serum was inactivated by heating in water bath one-half hour at 56° C.

The antigen was titrated by mixing it in varying amounts with one unit of the hemolytic system.

Preparation of antigen. After trying out several methods of antigen preparation it was finally determined that filtered autolysates gave the most stable and satisfactory products.

The antigens were prepared as follows: The organisms were grown on agar for forty-eight hours at 37.5° C., then taken off and suspended in distilled water and shaken for forty-eight hours in a mechanical shaker. This suspension was then heated at 56° C. for one-half hour, incubated twelve hours, after which enough sodium chloride and formalin was added to make an 0.85 per cent and 0.5 per cent solution respectively. Filtration was carried on through asbestos.

Preparation of immune serum. The same serums were used for this work as for the previous experiments, a description of which has already been given.

The results of the complement fixation tests may be seen in the following table:

ANTIGENS	ANTISERUMS					
	B. bron- chisepticus (dog) no. 36	B. bron- chisepticus (monkey) no. 123	B. bron- chisepticus (human)	Bact. per- tussis no. 0363	Bact. per- tussis no. 109	Bact. per- tussis no. 248
B. bronchisepticus (dog) no. 36	+	+	+	-	-	-
B. bronchisepticus (monkey) no. 123	+	+	+	+	+	+
B. bronchisepticus (human)	+	+	+	+	+	+
Bact. pertussis no. 0363	+	+	+	+	+	+
Bact. pertussis no. 109	+	+	+	+	+	+
Bact. pertussis no. 248	+	+	+	+	+	+

+ Denotes complete inhibition of hemolysis.

- Denotes incomplete or no inhibition of hemolysis.

It was found in a large majority of the tests as represented in the chart, which is a composite, that the *B. bronchisepticus* immune serum bound the complement in the presence of both the bronchisepticus and pertussis antigens, while the *B. pertussis* immune serum bound the complement in the presence of the homologous antigen and also the human and monkey strains of *B. bronchisepticus*. It did not bind the complement in the presence of a dog strain of *B. bronchisepticus*.

SUMMARY.

1. *B. bronchisepticus* immune serum bound the complement in the presence of both *B. bronchisepticus* and *Bact. pertussis* antigen.

2. *Bact. pertussis* immune serum bound the complement in the presence of *Bact. pertussis* antigen and *B. bronchisepticus* antigen of both human and monkey origin but not of dog origin.

3. The complement fixation test is not a reliable method of differentiating between the two organisms in question.

4. Bacterial autolysates were found to be the most stable and satisfactory antigens.

5. The complement fixation test was found to corroborate, in most respects, the agglutinin reactions reported in a previous paper.

REFERENCES.

- FERRY, N. S., AND NOBLE, ARLYLE. 1918. Studies relative to the apparent close relationship between *Bact. pertussis* and *B. bronchisepticus*. I. Cultural, agglutination and absorption reactions. *J. Bact., Balt.*, 3, 193.

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Studies from the Research Laboratory.

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**THE CONTROL OF LICE ON HORSES, WITH ESPECIAL
REFERENCE TO WINTER CONDITIONS.**

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In a previous paper dealing with horse lice, the writer (Hall, 1917) has noted that the problem of keeping horses free from lice becomes acute during the winter in the Temperate Zones whenever horses are exposed to infestation during part or all of the year. In the same paper some notes on the bionomics of lice were given, and some experiments along the line of control were mentioned, but not discussed. The object of this paper is to give the results of those experiments and some others.

The writer has recently encountered the idea that lice were formerly rather rare on horses in this country. Dr. E. M. Houghton, of this laboratory, states that he never saw them on horses during his boyhood on a farm. I am informed by my father that in all his experience as a cavalryman in the United States Army in Arizona he never saw lice on cavalry horses. On the other hand, the editor of an agricultural paper stated in 1916 that lice were often found on horses and mules in olden times, but are now seldom seen. Whether or not lice were more or less common in this country during the Civil War, and for some time thereafter, they are certainly sufficiently common pests at the present time. Among the horses purchased for the serum work of this company quite a number are found to be infested with lice. The same is true of horses being purchased at the present time for our army, as requests for information as to control measures indicate.

The control of lice in summer is a comparatively simple matter. Two treatments with a coal-tar dip, at 20-day intervals, afford a cheap and adequate treatment without toxic effects on the

horse, injury to the skin, or loss of hair. But winter weather is not suitable for this treatment under most conditions. The use of an aqueous solution of any sort chills the animal and increases the likelihood of pneumonia; the dip wets the horse, and wetting causes chilling. At times the objection may be overcome, as it is largely a matter of labor available to dry the animal after treatment. It is feasible to use coal-tar dips in winter, and to protect the animal against subsequent bad effects, where there are only a few animals to handle, or where there is an abundance of available assistance, as is the case in the army under ordinary conditions. The armies of the Allies, according to Haas, have dipped horses in a swim vat throughout the winter, the animals being dried and blanketed in a warm room when it was available, or being blanketed and taken to the stables when no such room was available. Something of the sort has been successfully accomplished under the direction of Dr. R. H. Wilson with the horses on serum production at Parkedale. This is a possible but not always attractive solution of the louse problem; it is not always a feasible solution.

Broadly speaking, insecticides may be applied to horses in one of five ways:

- (1) In aqueous preparations, which will wet the skin.
- (2) In volatile preparations, which will evaporate, wetting the skin only transiently and drying quickly.
- (3) In fatty or oily preparations, which will oil the skin, but not wet it.
- (4) In powders, which will not wet the skin.
- (5) In fumes, which will not wet the skin.

A good insecticide should be effective, cheap, easy to apply, cleanly, non-toxic to the animal, non-injurious to the skin, and should not cause depilatory effects.

A discussion of the advantages and disadvantages of the methods given above, and notes on experiments with some of them, are given in the following portion of the paper.

(1) Aqueous preparations, such as coal tar dips, are very satisfactory forms of treatment in summer, when wetting is not disadvantageous. In winter they must be used for choice on warm days, or in sheltering structures, and the animals should be quickly and thoroughly dried, rubbed down, and blanketed.

Failure to provide this care in winter may result in pneumonia. This is the best known and best established method of killing lice on horses, and is the method of choice wherever applicable. Since it is entirely satisfactory in summer and not in winter, eradication dipping in summer may be regarded as the best treatment for lice; this avoids the aggravated effects of lice infestations, and the apparent increase in number, together with the increased difficulties and danger to treatment in winter. With two dippings at suitable intervals (twenty days), dips of this type are satisfactory on practically all of the counts noted as desirable for an insecticide.

(2) Volatile preparations for killing lice are seldom used or recommended, but since they can be used in winter, owing to prompt evaporation, and since so little has been published along this line, the writer made some tests to ascertain at first hand some of the possibilities of such treatments.

Of the volatile substances, two which deserve early consideration as louse-killers are kerosene and gasoline. These substances have proved extremely effective against lice on man, and have been used undiluted with very little resultant dermatitis. They are comparatively cheap and easy to obtain. However, kerosene is inflammable, and is notoriously depilatory and irritant for horses, and gasoline is too inflammable and explosive to use around stables where hay and straw in abundance furnish material for a conflagration. Tests of kerosene mixed with equal parts of linseed oil were made on one horse, No. 2; no live lice were ever found on the horse in examinations during a month after treatment, but the linseed oil did not prevent the depilatory action of the kerosene or the production of dermatitis. A substance which is not depilatory and not explosive, though it is inflammable, is methyl alcohol (wood alcohol). This is known to have lethal and toxic properties, is easily obtained, and is comparatively cheap. It was therefore made the basis of most of the preparations tested.

The first of the methyl alcohol preparations tested was made by adding fluid extract of quassia (*Picrasma excelsa*) to methyl alcohol in the proportion of 3 ounces of the fluid extract to 30 ounces of alcohol, or 1 part to 10 by volume. The horse was sponged twice from head to tail with this preparation, and gone

over with a brush each time immediately after sponging. A number of the horses treated in this way had been rubbing, but no lice could be detected on them; others had lice, but were not critically examined after treatment, so that no conclusions as to efficacy could be drawn; but a number of animals found infested with *Hæmatopinus* were treated, and were subsequently examined to ascertain the efficacy of the treatment. Eight horses (Nos. 484, 517, 400, 601, 283, 409, 730, and 471) were treated for lice, though in some cases no lice were detected in spite of a history of the animal rubbing itself; these animals were not subsequently examined for lice, though casual examination in some cases indicated that the animals' coats were not injured by the treatment. Eight other horses (Nos. 173, 69, 746, 610, 57, 310, 706, and 457) were treated in spite of the fact that no lice were found on them; subsequent examination also failed to detect any lice, so no conclusions as to efficacy could be drawn; the animals' coats were not injured by the treatment. Eight others (Nos. 278, 491, 1,781, 452, 306, 677, 175, and 449) were found to have lice on preliminary examination, and were examined for lice after treatment. The results were as follows:

Horse No. 278 had many lice. For several days after treatment dead lice were noticed on the animal, and an examination on the ninth day after treatment disclosed only one live louse. Fourteen days after the first treatment a second was given. Thirty-six days after this second treatment the animal was carefully examined, and no live lice were found.

Horse No. 491 had many lice. Nine days after treatment no live lice were found. It was examined again fourteen days after treatment, and no lice were found. The treatment was repeated on this day. It was examined twenty-one and thirty-six days after the second treatment, and no lice found on either occasion.

Horse No. 1,781 had lice. Thirty eight days after treatment no lice were found.

Horse No. 542 had many lice. Twenty-one days after treatment no lice were found. Thirty-six days after treatment one live louse was found.

Horse No. 306 had many lice. Twenty-one days after treatment one young louse was found. Thirty-six days after treatment none were found.

Horse No. 677 had many lice. It was examined twenty-one days and thirty-six days after treatment, and no lice were found.

Horse No. 175 had lice. It was examined twenty-one and thirty-six days after treatment and no lice found.

Horse No. 449 had lice. It was examined thirty-six days after treatment and no lice found.

Of these eight horses, five had large numbers of lice, and three were lousy enough to make the finding of lice on them before treatment an easy matter. In eleven examinations after treatment no lice were found; in two examinations, at intervals of twenty-one and thirty-six days after treatment, one louse was found in each case, one of them being evidently a young louse which had hatched subsequently to treatment. This appears to be conclusive evidence that the treatment will kill lice present on the animal. It is possible that the treatment exerts a detrimental effect on the louse eggs, but it is evident that as administered some eggs escape this effect and hatch. Probably two treatments at a twenty-day interval, if properly given, would completely free a horse from lice, so that it may be regarded as a fairly satisfactory control measure for lice in winter where dipping is not feasible.

A second methyl alcohol preparation was made with methyl alcohol as a base and contained 10 per cent. fluid extract of quassia, as in the foregoing preparation, and also 5 per cent. glacial acetic acid, and 1 per cent. coal-tar creosote oil. Two horses, Nos. 339 and 1,218, were treated with this preparation. No lice were found at the time of treatment, or seven or forty days after treatment, so there were no conclusions as to its efficacy, but the treatment had no bad effects on the animals or on the skin or hair.

A third methyl alcohol preparation was made with 20 parts methyl alcohol, 2 parts glacial acetic acid, and 2 parts coal-tar creosote oil. Two horses, Nos. 55 and 396, were treated with this preparation as follows:

Horse No. 55 had many lice. Seven days after treatment it still had many lice and the coat was roughened. Thirteen days after treatment, it still had many lice, and was then treated with plain methyl alcohol. Twenty-seven days later no lice were found on examination, and the coat was smooth. The methyl alcohol to

which other ingredients had been added, as noted, seemed inferior to methyl alcohol alone and exerted an injurious effect on the coat.

Horse No. 396 was examined for lice, but none were found. It was given the same treatment as was given No. 55 the first time; seven days later no lice were found, but the coat was rough. Forty days after treatment no lice were found, and the coat was again smooth. No conclusions as to efficacy in this case.

The mixture used seems to be unsatisfactory as regards efficacy and effect on the coat.

A fourth methyl alcohol preparation was made with 400 parts methyl alcohol, 20 parts glacial acetic acid, and 5 parts coal-tar creosote oil.

Eight horses were treated with this as follows:

One horse, No. 24, was examined for lice, but none were found. It was given the treatment noted above. Seven days later no lice were found, but the coat was roughened. Forty days later no lice were found, and the coat was smooth.

Seven horses had lice and were treated with the above preparation with the following results:

Horse No. 209 had lice. Seven days after treatment no live lice were found, but the coat was roughened. Forty days after treatment, one live louse was found; the coat was smooth.

Horse No. 312 had lice. Seven days after treatment no lice were found, but the coat was roughened. Forty days after treatment no lice were found, and the coat was smooth.

Horse No. 303 had lice. Seven days after treatment a young louse and one older louse were found. The coat was roughened. Thirteen days after treatment one young louse was found. About this time the treatment was repeated. Forty days after the first treatment the horse was examined and no lice found; the coat was smooth.

Horse No. 9 had many lice. Seven days after treatment no lice were found. The coat was roughened. Forty days after treatment two lice were found. The coat was smooth.

Horse No. 490 had lice. Seven days after treatment no lice were found. The coat was roughened.

Horse No. 365 had lice. Seven days after treatment no lice were found. The coat was roughened.

Horse No. 1758 had lice. Seven days after treatment no lice were found. The coat was roughened. Forty days after treatment two lice were found. The coat was again smooth.

In the foregoing tests of this fourth methyl alcohol preparation, six of the seven horses had lice enough to find easily, and the seventh had a heavy infestation. In thirteen examinations after treatment no lice were found in eight examinations; one louse was found in each of two examinations, and two lice were found in each of three examinations. Of the eight lice found after treatment two were evidently young lice, and it is quite possible that some of the other lice had hatched after treatment. The coat was rough a week after treatment and smooth forty days after treatment.

In the cases already noted in this paper, the lice were *hæmatopinus*. *Trichodectes*, as already noted (Hall, 1917), was only found on two horses, in both cases in heavy infestations. The lice were destroyed on one horse, No. 772, as previously published, by the application of sodium fluoride. The other horse, No. 773, was sponged, as described, with methyl alcohol. Fifteen days after treatment no lice were found.

Sponging with methyl alcohol, alone or with the addition of fluid extract of quassia, or of glacial acetic acid and coal-tar creosote oil, is a fairly effective control measure for lice on horses in winter. It must be applied thoroughly and carefully, but if so applied it is likely that two treatments at a suitable interval, twenty days, would completely free a horse from sucking or biting lice. The methyl alcohol is somewhat unpleasant to handle, and the fact that it is inflammable must be kept in mind.

Some experiments *in vitro* covering volatile substances primarily, but incidentally some non-volatile ones, were carried out as follows:

Lice were taken in lots of eight or more and momentarily immersed in the fluids noted below; the immersion being just long enough to wet the lice and the horse hairs to which they clung; they were then lifted out and placed with the hairs in a bottle. This was incidental work, which was interrupted at times by more urgent matters, and there are wide breaks in some of the observations.

HEMATOPINUS ASINI.

Kerosene.—No sign of life after dipping.

Kerosene, 10% ; *Methyl alcohol*, 90%.—Some alive 1 hour and 35 minutes later ; all dead 19 hours later.

Glacial acetic acid, 10% ; *water*, 90%.—Some alive 8 minutes later ; all dead 19 hours later.

Glacial acetic acid, 25% ; *water*, 75%. No sign of life after dipping.

Glacial acetic acid, 50% ; *water*, 50%.—No sign of life after dipping.

Glacial acetic acid.—No sign of life after dipping.

Glacial acetic acid, 10% ; *methyl alcohol*, 90%.—One louse alive 3 hours and 35 minutes later, others dead ; all dead in 13 hours.

Glacial acetic acid, 5% ; *methyl alcohol*, 94% ; *coal-tar creosote oil*, 1%.—No sign of life after dipping.

Methyl alcohol.—No sign of life after dipping.

Methyl alcohol, 90% ; *fluid extract of quassia*, 10%.—No sign of life after dipping.

Fluid extract of quassia, 10% ; *water*, 90%.—All alive an hour later ; all dead in 22 hours.

Coal-tar creosote dip (*Kreso dip No. 1*), 1% ; *Methyl alcohol*, 99%.—No sign of life after dipping.

Coal-tar creosote dip (*Kreso dip No. 1*), 1.6% ; *water*, 98.4%.—All alive 5½ minutes later ; all dead in 21 hours.

Liquid petrolatum (*American oil*).—Lice moved for a few moments and gave no sign of life afterwards.

Checks.—Kept at same temperatures, 21-24.5° C. ; all dead in 23 hours.

TRICHOECTES PILOSUS.

Methyl alcohol, 90% ; *fluid extract of quassia*, 10%.—No sign of life after dipping.

Coal-tar creosote dip (*Kreso dip No. 1*), 1% ; *methyl alcohol*, 99%.—One alive in 2 hours and 50 minutes ; others dead ; all dead in 21½ hours.

Coal-tar creosote (*Kreso dip No. 1*), 1.6% ; *water*, 98.4%.—One alive 30 hours later ; all dead in 45 hours.

Fluid extract of quassia, 10% ; *water*, 90%.—Four alive 48

hours later; one alive 147 hours (over 6 days) later; all dead in 7 days.

Liquid petrolatum (American oil).—Lice moved for a few moments and gave no signs of life afterward.

Checks.—Some were alive at the end of 103 hours; all dead in 120 hours.

A consideration of these experiments indicates that *Hæmatopinus* only lives a short time off its host, regardless of insecticidal treatments applied, so that unless an insecticide kills rapidly, little can be inferred. Some insecticides are extremely effective, but slow in their action, and sucking lice die too soon to permit of conclusions in regard to the action of such insecticides.

Of the substances tested on *Hæmatopinus*, the following killed immediately or almost immediately: Kerosene; glacial acetic acid in strengths of 25, 50, and 100%; glacial acetic acid, 5%; methyl alcohol, 94%; coal-tar creosote oil, 1%; methyl alcohol; coal-tar creosote dip (Kreso dip No. 1) 1%, methyl alcohol 99%; methyl alcohol 90%, fluid extract of quassia 10%; and liquid petrolatum (American oil). It is practically impossible to draw conclusions from the other experiments, except to the effect that the substances used are not immediately or rapidly lethal for *Hæmatopinus* when subjected to momentary immersion in them.

Of the substances tested on *Trichodectes* the following killed immediately, or almost immediately: Methyl alcohol, 90%, fluid extract of quassia, 10%; and liquid petrolatum (American oil). The following were evidently effective, but slower in their action, the relatively long time between immersion and death resulting from shortening the time element in the immersion: Coal-tar creosote dip (Kreso dip No. 1) 1%, methyl alcohol 99%, and coal-tar creosote dip (Kreso dip No. 1) 1.6%, water 98.4%. Fluid extract of quassia was evidently devoid of insecticidal action on *Trichodectes* as used (*i.e.*, by momentary immersion), for the insects treated outlived their checks.

It is rather surprising to find that either kerosene or methyl alcohol was immediately lethal for *Hæmatopinus*, but a mixture of 10% kerosene and 90% methyl alcohol was much less effective, some lice being alive an hour and 35 minutes later. Similarly, it is surprising to find that 90% methyl alcohol with 10% fluid extract of quassia (fluid extract of quassia in the experi-

ment with *Trichodectes* being ineffective in momentary immersion) was immediately lethal, whereas 90% methyl alcohol with 10% glacial acetic acid (glacial acid being immediately lethal in 25 % solution) left one louse still alive 3 hours and 35 minutes later.

Not too much may be concluded from the foregoing experiments, but everything indicates that methyl alcohol may be used successfully in lice control in winter. The horse should be sponged with a liberal amount of alcohol, then brushed, then sponged and brushed again. If this work is not done carefully, the failure to get results will show it. While the alcohol may exert a detrimental effect on the louse egg, and probably does, an effective exposure of the eggs to the alcohol can hardly be assured by sponging, and two treatments at 20-day intervals would be indicated for eradication measures.

(3) Fatty or oily preparations are not infrequently recommended for freeing horses from lice. They are objectionable on account of the fact that such preparations are rubbed off the horse on to the stall, harness and everything else with which the horse comes in contact, and if the persons who have occasion to handle the horse escape soiling their clothing from the horse direct, they will usually succeed in soiling it from contact with these objects. Furthermore, fats and oils are frequently depilatory for horses and must be used with caution. Why a horse is so prone to lose hair on apparently trifling provocation does not seem to be well known, but it is well known that they lose it. In this connection it will be noticed that tests *in vitro* showed liquid petrolatum (American oil) to be very effective against both species of lice. Lest someone should test liquid petrolatum in practice as a louse killer, I hasten to add that it is also depilatory. My attention was called to the fact that liquid petrolatum is depilatory by Dr. David Buckingham, of Washington, D.C., about two years ago, and I have confirmed the fact by test since.

The only oily dressing I have tested to any extent as a treatment for lousiness in the horse, is horse fat. Kirk (1917) recommended this fat for oily dressings in very bad cases of mange, as a preliminary to dipping in a calcium sulphide dip. He states: "I am certainly sure that an oily dressing does blister the skin, no matter what oil is used—with one exception—and that is

the oil obtained from the boiling down of carcasses of the horse. The fat obtained in this way appears to have not the least derogatory effect on the skin, but on the contrary is of very great value, and it can be confidently recommended." Kirk has nothing to say in regard to using this horse fat to combat lice, but as it appears from investigations in the control of lice on man that such substances as petrolatum are substantially as effective as medicated preparations having petrolatum as a base, the action being primarily mechanical and consisting in coating and suffocating the louse, it seemed evident that horse fat would kill lice, and, according to Kirk's report, would not injure the horses' coats.

We tested horse fat, or a mixture of 4 parts horse fat and 1 part linseed oil, containing 3 per cent coal-tar creosote oil, on fourteen horses, the weather at the time being very cold, sometimes above zero, and sometimes below, but seldom much above zero. The fat was obtained from a rendering tank and had a melting point around 25° C. (77° F.). The fat was melted by immersion in a container in water heated by steam until the fat was fluid, and the melted fat was applied to the horse by means of sponges or wads of cotton batting. During the cold weather then prevailing the fat on the ends of the hair would promptly chill out and solidify soon after the animals were returned to the barns, the chilled fat pasting clumps of hair together in quills. When this was brushed or combed, fat from the warmer region, close to the body, would slowly flow out on the hair and solidify in turn. The quilling effect exposed the skin to the cold, with the result that these horses would shiver in cold weather when other horses were standing still and showing no indications of being cold. Offhand one would suppose that oil or fat on the body would aid in keeping it warm, but experience proved that this was not the case, the reason being apparently the quilling or clumping of the hairs and the consequent exposure of the skin between the hair clumps.

Of the fourteen horses treated with horse fat, six were not groomed for several days after treatment, the idea being that the fat would not injure the coat and that it would serve to kill young lice as they hatched, provided they hatched. These horses suffered depilatory effects, some of them losing large amounts of

hair, contrary to what Kirk's recommendations had led us to expect. The other eight horses were groomed the day after treatment and the fat removed daily with comb and brush and rag as fast as it flowed down the hairs and congealed. These horses did not suffer depilatory effects

To test out the apparent fact that horse fat would not cause depilatory effects where it was not allowed to remain too long, four additional horses were treated. As soon as the first animal was treated the fat was scraped off as completely as possible with sweat-scrapers. A second animal was treated, and then curried immediately. A third animal was treated, and sent back to the barn with instructions to curry it promptly and as often as necessary to keep down the accumulation of fat on the hairs. A fourth animal was treated and then washed down with methyl alcohol to remove the excess fat; this was not a very satisfactory way of removing the fat. All horses were curried daily. None of them suffered depilatory effects. The use of the sweat-scraper proved the most satisfactory method of removing the excess fat and preventing depilation. Apparently the excess fat exerts a "smothering" effect on the hair follicles. This is not intended as a lucid and scientific explanation of the loss of hair; it is, however, substantially as lucid, scientific, and plausible as most of the explanations for bald-headedness in men.

Horse fat proved entirely effective as a louse killer. It had some effect on the eggs, as only seven hatched out of several dozen tested after removal from horses treated with the fat, and none hatched out of forty-seven removed from a horse after treatment with the horse-fat linseed oil creosote oil mixture.

The eggs in both lots looked abnormal. They were discolored, shriveled, or contained air bubbles, as a rule. It seems quite reasonable that hardening films of fat would seal the embryo away from its necessary oxygen supply, and perhaps seal the lid of the egg against efforts of the young louse to escape from the shell.

(4) Powders are sometimes used against lice, but with the exception of sodium fluoride, which the writer (Hall, 1917) found entirely effective against biting lice, and entirely ineffective against sucking lice, powders are usually unsatisfactory. The effectiveness of ordinary louse powders is largely a function of

those constituents which are simultaneously volatile and toxic. The toxic effectiveness of volatile substances is largely determined by the degree of concentration of the volatilized material, and in applying a powder of the sort to a horse one is working under a set of circumstances which make for ineffectiveness. A powder which is fundamentally good will kill some lice and stupefy others, but the latter will recover as the material capable of volatilizing is driven off by the heat of the animal's body. Experiments and experience show that the idea that finely pulverized material clogs the breathing apertures of insects and so mechanically suffocates them is not tenable, or the actual injury so accomplished is usually very slight.

Inasmuch as naphthalene is one of the most effective of the powdered insecticides, being the principal constituent of many insect powders, and practically the sole constituent of "moth balls," a test was made of a mixture of approximately equal parts of naphthalene and plaster of Paris, horse being covered with the mixture, then covered with gunny sacks with the mixtures rubbed into them, and finally blanketed for 20 minutes to hold the volatilized naphthalene as much as possible. The horse was said to be lousy, but we saw no lice. Nine days later the horse was examined and a number of lice found.

Another horse was tested with a mixture made up on the formula of the "Lawry lice powder," but substituting a coal-tar creosote oil for the crude carbolic acid in the following formula: Mix 3 parts of gasoline with 1 of crude carbolic acid, and stir in enough plaster of Paris to make a dry powder. This mixture was applied to a lousy horse and the horse blanketed for half an hour. Nine days later the horse was still lousy.

In the control of lice on man, louse powders have proved to be of more value as palliative measures than as eradicated measures. They appear to be of service principally in tiding over individuals who become infested under conditions that do not permit of adequate insecticidal treatment at the time. Since this is the case in regard to lice infestations on man, where the clothing can be utilized to hold the powder and its vapors, still less success may be anticipated in lice infestation on horses where the vapors from volatile constituents can pass readily through the short coat of hair and escape.

(5) The application of insecticides in the form of fumes to horses is hardly feasible under the ordinary conditions of veterinary practice, as some sort of fumigation chamber must be provided in order to expose the body of the horse to the fumes and at the same time to provide the horse with air free from toxic fumes to breathe during the fumigation process. However, under conditions where the number of horses and the need of treatment warrant it, this method of treatment may be used. It has been used during the present war in the control of mange in French horses, and the fumigant used there in the control of mange, sulphur dioxide, has also been found adequate for the control of lice. The writer has never tested fumigation as a control measure for lice.

I take pleasure in acknowledging the friendly co-operation and assistance of Dr. R. H. Wilson and Dr. L. A. Maze in carrying out these experiments.

SUMMARY.

The best control measure for lice on horses is eradicated dipping in summer. There are numerous aqueous solutions that are satisfactory, the ones in most common use being coal-tar dips. These preparations are effective, uninjurious, comparatively cheap, and readily obtainable. Their use in winter is feasible at times, but is limited by the danger of chilling, and consequent production of pneumonia. Eradication dipping calls for two dips at a 20-day interval.

Of the volatile substances that may be used for lice control in winter, methyl alcohol seems to be the most satisfactory of the things tested, as it is effective, is not too expensive; and does not injure the hair or coat. It is inflammable and somewhat unpleasant to handle. It cannot be depended on to kill eggs, so two treatments at a 20-day interval are indicated.

Of the fatty or oily substances that may be used for lice control in winter, horse fat appears to be a fairly cheap and satisfactory representative. It should be melted and applied, and the excess promptly scraped off with a sweat-scraper. After treatment the horses should be thoroughly groomed daily to remove the fat which flows to the tip of the hairs. The treatment has the disadvantage of greasing the clothing, stalls, harness, and other

things with which it comes in contact. Contrary to what one might expect, horses so treated will be colder in very cold weather than horses not treated.

It is non-irritant, but if it is left on and the horses are not groomed for several days, it has a depilatory action. It appears to be injurious to eggs, but some eggs will hatch after this treatment so that eradication would call for a second treatment at a 20-day interval. Most oils, whether fixed or volatile, are depilatory for horses, and the volatile oils are commonly irritant as well, producing dermatitis.

Powders do not appear to be very satisfactory substances for the control of lice on horses. They are not especially effective on man, where conditions are better and where many tests of various preparations have been made. They apparently exert no effect on the eggs and can only be regarded as palliative, killing a few lice, and temporarily stupefying or disturbing others.

Fumigation with sulphur dioxide appears to be a dependable measure for the control of lice on horses, but it has only limited application. It appears to be a useful measure in the army, where it is intended primarily as a treatment for scabies.

REFERENCES.

- Hall, Maurice C., 1917. "Notes in Regard to Horse Lice." *Jour. A.V.M.A.*, v. 4 (4), July, pp. 494-504, 3 figs. 1918. *Idem. Vet. Jour.*, v. 74 (3), March, pp. 87-95, 3 figs.
 Kirk, Hamilton, 1917. "Skin Diseases of the Horse." *Vet. News* (710), v. 14, Aug. 11, pp. 320-323, figs. I-II, IV.

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THE EFFECT OF THE X-RAY UPON THE RESPONSE OF TADPOLES TO THYROID STIMULATION.

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INTRODUCTION.

The experiments carried out by Gudernatsch¹ in 1912 upon the growth of tadpoles after thyroid gland feeding, have been repeated in this laboratory during four summer seasons. In short, Gudernatsch observed that tadpoles whose normal metamorphosis into frogs occupied from three to six months, completed this metamorphosis in five to ten days when small quantities of thyroid gland substance were added one or more times to the living water of the tadpoles. This induced early differentiation into frogs, including all the gross changes normally distributed over several months. The miniature frogs so produced, although apparently anatomically perfect, were unstable and regularly died after a few days.

In all essential respects our findings have been in accord with Gudernatsch's reports. The experiments of this laboratory were extended to include low life forms other than tadpoles and also tadpoles of certain frog species whose metamorphosis normally extends over two or more years. An effort was made to determine the influence of thyroid feedings upon the growth and differentiation of tadpoles whose natural development had been deviated by diverse procedures.

The present report concerns itself with observations made as to the development of thyroid-fed tadpoles which had been exposed to the action of the x -ray.

Three distinct theories have been advanced to explain the action of the Roentgen ray upon normal tissue cells. One of these, adhered to by O. Hertwig² and his coworkers (1912), is that the rays exert a specific destructive action upon the chro-

matin of the cells. Opposed to Hertwig's hypothesis is the older theory proposed by Schwartz³ (1903), who concludes from the observation of the destructive action of the Roentgen ray upon the lecithin of egg yolk, that the injury to the cells is due to the destruction of the lecithin which they contain.

Richards⁴ (1914-15) has shown that the activity of various enzymes or ferments, of both animal and vegetable origin, is susceptible to change through the influence of Roentgen rays. He concludes from his experiments that "a short radiation has the effect of accelerating the activity of these enzymes (diastase and pepsin), while longer radiation is inhibitive. Between these strengths lies a point at which the radiation is non-effective." His observations on pepsin and diastase have since been extended to include other enzymes, the results all tending to confirm his original conclusions. He advances the theory, therefore, that "life processes are subject to marked change under the influence of radiation, a slight exposure being accelerative in most cases, while a more intense treatment is inhibitive or destructive. As a causal factor in these effects, the demonstrable injury to the chromatin of the cell is undoubtedly important; but there are also good evidences that the modifiability of enzymes under the action of the rays likewise plays a considerable part either directly or indirectly in the resulting injury."

MATERIALS AND METHODS.

The tadpoles collected to participate in these experiments conformed within the acceptable limits in weight, length and in stage of development. These were maintained in large shallow trays under good condition as to light, air and aeration of living water. The food, apart from thyroid gland materials, consisted of green algae supplemented at intervals with small quantities of desiccated beef liver.

Tadpoles of the species *Rana catesbiana* (bull-frog) exclusively were used. The individuals of this species normally live for at least two years in the larval state. This fact permitted the use of larvae representing two widely different stages of development. The younger group (Experiment A) was composed of tadpoles approximately one year old. At this stage the individuals exhibited no signs of differentiation into the adult form.

The second group (Experiment P) was composed of tadpoles not quite two years old. These tadpoles would under the natural conditions have completed their metamorphosis before the end of the summer. The controls in this experiment actually did complete their metamorphosis, but long after the conclusion of the experiment.

In both experiments (1st and 2nd year groups) the tadpoles were divided into four equal and similar lots. Two lots in each experiment were then subjected to the rays from a Coolidge tube according to the following standardized formula:*

	Experiment A.	Experiment P.
Current	6 milliamperes	12 milliamperes
Spark Gap	8 inches	7 inches
Distance from Anode.....	12 inches	12 inches
Time	2½ minutes	4 minutes

Tadpoles of Experiment A were irradiated a second time one week later, under identical conditions except that the current was doubled.

In each experiment one irradiated group and one untreated group were placed on thyroid feedings while the second irradiated group and the second control group were kept on the desiccated liver and algæ diet. The thyroid was administered by scattering 100 mgms. of the commercial desiccated product in the living water.

After the death or complete metamorphosis of all the thyroid-fed tadpoles of Experiment A, and tabulation of the accrued data, the two remaining control groups were again divided. Half of each group was placed on thyroid treatment identical with that described above. This second feeding was started one month after the irradiation of the tadpoles.

The alterations in size and shape of the tadpoles incident to their development were recorded by means of a method of "shadow photography." This procedure afforded a permanent and relatively accurate record of the growth. For the purposes of carrying out this photographic mensuration a temporary dark room was constructed near the tables containing trays of tadpoles. In this dark room was placed a small shallow rectangular glass container (2"x4") with a smooth flat bottom. Photographic printing paper was cut in such size as to fit exactly into the

*The tadpoles were irradiated by Dr. George C. Chene, Detroit. Our thanks are due him for his courtesy.

rectangular container. One piece of this paper was placed in this container sensitive side up. Superimposed upon the sensitive paper was a mask of transparent celluloid upon which had been printed lines at millimeter intervals. Representative tadpoles to be measured were taken from their trays, placed in small beakers in a constant quantity of clear water. To this was added a few drops of chloroform (two to ten, depending upon the size of the tadpoles) to prevent their moving while being photographed. As soon as the tadpoles were motionless they, together with the water, were poured into the rectangular chamber previously prepared. Directly above the chamber, at about the height of 18 inches, a high power nitrogen electric lamp was placed to whose light exposure was made for two or three seconds. The motionless tadpoles resting on the millimetered mask served as a negative and the linear dimensions were directly printed out on the sensitive paper. The chloroform was found to be harmless for all except very young tadpoles. It is necessary, however, that immediately after being photographed the tadpoles be removed from the chloroform water. This method of recording dimensions is much less tedious than actual photography and is more rapid and less irksome than the actual measurement with dividers, at frequent intervals, of many hundreds of tadpoles.

TECHNICAL DATA AND COMMENTS.

The effect of thyroid feeding on normal tadpoles varies with the age of the individuals. This is to be expected in view of the nature of the changes induced. In our experiments with two-year-old tadpoles (*Rana catesbiana*) which had reached the stage in development immediately preceding the initiation of metamorphosis, thyroid feeding served simply to induce a premature and accelerated differentiation. Thyroid-treated tadpoles differentiated into normal frogs, wholly indistinguishable from the controls.

In the case of one-year-old tadpoles, thyroid feeding was invariably followed by the death of the individuals. Certain somatic changes occurred before death, which were obviously similar to those changes which take place in the metamorphosis of older individuals. The alterations which were macroscopically observable occurred in approximately the following order:

1. Marked diminution in general size.
2. Alteration in the shape of the body, the rounded, well-fed larval shape giving way to the slender, trim adult form.
3. Elevation of the eye-balls above the dorsal surface.
4. Increase in width and size of the mouth.
5. Acceleration of growth of hind legs.
6. Thinning and eventual breaking through of the ventral body wall at points corresponding to the future site of the fore legs.
7. Noticeable growth of the fore leg buds.
8. Disappearance of the "fin" from the tail.
9. Shortening of the tail.

It was observed that the tadpoles all reached a certain stage in this abnormal metamorphosis, at which point death occurred. The examination of a large number of specimens preserved shortly after death demonstrated that all were very similar in the extent to which the changes outlined above had occurred, and that the date of death affords a valuable criterion as to the reaction of the tadpole to thyroid treatment. This fact has been noted before and other workers have taken the time of death of the treated tadpoles as the end-point in the quantitative estimation of thyroid activity. (Marine and Rogoff, 1916; C. H. Lenhart, 1915.)

The following table indicates that in experiment A (one-year-old tadpoles) the irradiated tadpoles reacted earlier than did those that were unexposed. At the outset of the experiment equal numbers (17) of tadpoles composed each of the four lots to be compared. The normal tadpoles (A) and x-rayed tadpoles (A₂X) which were thyroid fed responded to thyroid stimulation and died upon attaining to a certain phase of metamorphosis; however, the x-rayed tadpoles earlier attained to this point.

TABLE I.

DATE	A Thyroid	A ₂ X Thyroid plus X-Ray	A ₂ Control	A ₁ X Control plus X-Ray
5-20	17	17	17	17
5-21	17	11	17	17
5-24	(17) 14*	8	17	17
5-25	11	4	17	17
5-26	4	1	17	17
5-29	4	0	17	17
5-31	3	0	17	17
6-1	2	0	17	17
6-3	0	0	17	17

*Three killed for photographing.

The first death in the non-rayed (A_1) group occurred four days after the first death in the irradiated group, or when the irradiated tadpoles had been reduced to a fourth of their original number. So also the last survivor in the non-rayed group lived five days longer than did the last of the irradiated group. The exposure of tadpoles to the action of x-rays in some unknown manner determined an increase in the rapidity of their reaction to thyroid stimulation.

This conclusion is supported by photographic evidence. In figure I are shown views of average individuals from groups A_1 and A_2X . (Thyroid-fed and thyroid-fed, x-rayed, respectively.) These photographs were made on the same day and under identical conditions of focus, and of distance of the object from the lens.

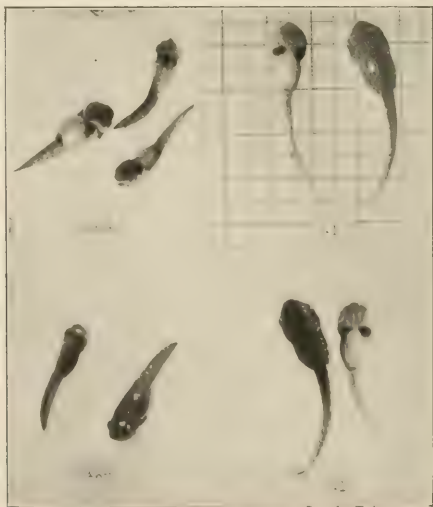


FIG. 1

Figure I. Photographs made at the same time of average tadpoles from group A_1X (thyroid-fed and X-rayed) and from group A_1 (thyroid-fed but not exposed to X-rays). The relative size of the tadpoles may be estimated from the millimeter lines included in this one photograph.

The metamorphosis of the individuals in A_2X is obviously further advanced than those in A_1 . This is indicated by the following points:

1. Smaller size.
2. More triangular shape.
3. More prominent eye balls.
4. Larger mouth.
5. Shorter tail.
6. Presence of fore-leg buds (discernible as small white spots in the center of the dark area of skin rarefaction). Under the

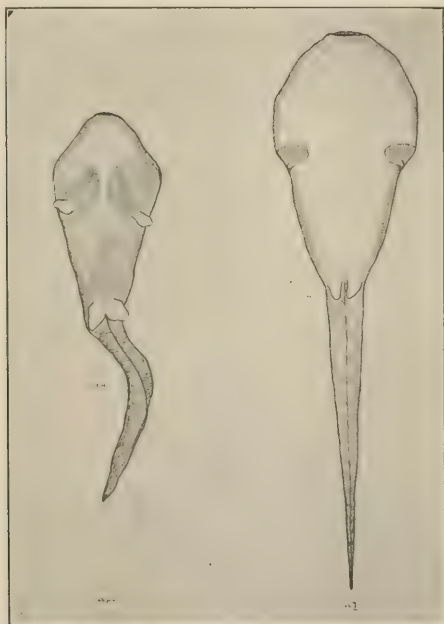


FIG. 2

Figure II. Drawings three times natural size of average tadpoles, illustrating the differences in the degree of metamorphosis attained at a certain time by members of groups A_1 and A_2X , respectively.

binocular microscope fore leg buds were also discernible on the tadpoles of A_1 . They were much smaller, however, than on the other tadpoles.

Groups A_1 and A_1X , which had not been thyroid-fed and which had exhibited none of the phenomena of metamorphosis, were now divided and half of each group placed on thyroid feedings. As before, the x-rayed tadpoles began to differentiate before the unexposed individuals showed any signs of change. The difference was not so marked in this case, undoubtedly because of the time (30 days) which had elapsed since exposure to the x-rays. It is significant that the irradiation was, in some measure, effective after such an interval of time.

It might be maintained that the changes induced in young tadpoles by thyroid feedings are so abnormal as to furnish no dependable index to their reactivity. For this reason tadpoles which would normally metamorphose within the space of a few months were subjected to the same treatment.

Table II gives the relative condition of the tadpoles July 2nd, at which time the thyroid-fed tadpoles were in the midst of the metamorphosis process. The results are in accord with those already outlined.

TABLE II.

State of Metamorphosis	P_1 Thyroid	P_2 X-Ray Thyroid	P_3 X-Ray	P_4
Number of tadpoles (7-2)	12	5	11	19
Number of tadpoles with 4 legs	2	2	0	0
Number of tadpoles with 3 legs	2	1	0	0
Number of tadpoles with 2 legs	7	1	11	19
Per cent of tadpoles with 4 legs	16.6%	40%	0%	0%
Per cent of tadpoles with 3 legs	16.7%	20%	0%	0%
Per cent of tadpoles with hind legs only	66.3%	20%	100%	100%

In the tadpoles entering into this experiment, no developmental differences were detectable in group P_3 , which had been x-rayed, and in group P_4 , which were normal. That is, the x raying of the tadpoles brought about no demonstrable alteration in growth-differentiation processes. Both the x-rayed and normal tadpoles completed their metamorphosis at the anticipated time. Also, no differences were observable in the rate of response to thyroid feeding in the x-rayed tadpoles, group P_2 , and the non rayed thyroid fed tadpoles, group P_1 . The metamorphosis of both groups was hastened, but equally so, individuals composing both groups completed their metamorphosis and survived. In so far

as the results of observation on this small number of test animals permits, it is inferred that the exposure to the *x*-ray exerts no gross effect upon the process of metamorphosis, either in normal tadpoles or in thyroid-fed tadpoles.

The observation that the irradiation is without demonstrable effect upon normal tadpoles is important in considering the mechanism of the reaction produced upon thyroid-fed tadpoles. Several observers have shown that following intensive exposure to *x*-rays there may be found a slight increase in nitrogen metabolism of normal animals. (Baermann and Linser,⁵ 1904, Benjamin and Van Reuss,⁶ 1906.) If the results recorded in this paper are due to the direct action of the rays upon metabolism, the same phenomenon also occurs in those tadpoles which received no thyroid material. The occurrence of changes in the metamorphosis of the thyroid-fed groups only, is an indication that these changes are due to an altered susceptibility on the part of the tadpoles to the thyroid hormone.

This interpretation is apparently in keeping with Richard's theory of the mode of action of the Roentgen rays. He states that a small dose of the rays serves to increase the activity of certain, and presumably of all, enzymes, while large doses produce the opposite effect. In our experiments, weak irradiation of the tadpole increased the activity of a normal hormone (thyroid), administered subsequent to the irradiation. According to Richard's theory, strong irradiation should decrease the activity of the hormone when given under identical conditions.

In the event that further experiments prove that large doses of *x*-rays produce an effect opposite to the results here recorded, it may be inferred that the thyroid hormone normally acts in conjunction with the intracellular enzymes to produce the phenomena commonly associated with thyroid activity. Such an interaction has often been postulated by writers on the thyroid gland, but experimental evidence has hitherto been lacking.

SUMMARY.

Selected tadpoles were subjected to the action of Roentgen rays in small amounts. Certain individuals were then treated with preparations of thyroid gland and the rate of their metamorphosis compared with the metamorphosis rate of (1) normal

tadpoles, of (2) thyroid-fed tadpoles which had not been irradiated, and of (3) irradiated but not thyroid-fed tadpoles. The results of our experiments indicate that irradiation is without apparent effect upon normal tadpoles, but determines a slight but distinct increase in the susceptibility of young tadpoles to thyroid stimulation.

REFERENCES TO LITERATURE CITED.

- (1) Gudernatsch (J. F.) Arch. f. Entwickmech. d. Organismen., 1912, 35, 457.
- (2) Hertwig (O. G.) and (P.) Arch. f. Mikr. Anat., 1911, 77, 2. Abt., 1-95, 4 pl.
- (3) Schwarz (G.) Arch. f. Physiol., 1903, 100, 532.
- (4) Richards (A.)
 Am. Jour. Roent., 1915, 2, 908.
 Am. Jour. Phys., 1914, 35, 224.
 Ibid, 1915, 36, 400.
- (5) Baermann (G.) and Linser (P.) Munchen. Med. Wehnschr., 1904, 51, 996.
- (6) Benjamin (E.) and v. Reuss (A.) Munchen. Med. Wehnschr., 1906, 8, 1862.

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TWO NEW FLUKES FROM THE DOG.*

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So far as we are aware, the only fluke reported from the dog in the United States is *Paragonimus kellicotti*. This fluke occurs in the lungs of dogs, cats and swine. Ward and Hirsch (1915) note that the worm was discovered in the lung of a dog from Ohio by Kellicott. They also note, regarding lung fluke: "Null stated casually that it occurs in dogs and cats from the Oriental quarters of San Francisco, but gave no further data regarding the parasites."

In a series of 300 dogs examined post mortem here at Detroit, we have found intestinal flukes in 7 animals (Nos. 122, 133, 134, 195, 229, 231 and 281). The flukes of these dogs belong to 2 different species. The 2 species of flukes appear to be undescribed, and we have accordingly created new species for them.

An examination of our flukes shows that they belong in the genus *Hemistomum*, but an examination of the status of this generic name, which is the one in common use, indicates that the name is not in good standing, and while we are reluctant to tamper with nomenclature, a proceeding which always invites criticism from some school of zoologists, we are even more reluctant to refer new species to genera which are without standing. According to Stiles and Hassall (1908), *Hemistomum* Diesing, 1850, has as its type species, by inclusion and by the first species rule, *H. alatum* (Goeze, 1782) Diesing, 1850. But Railliet (1896) has renamed *Hemistomum* Diesing, 1850 (not Swainson), proposing

*Read before the Zoology Section of the Michigan Academy of Science, March 29, 1918.

the name *Conchosomum* Railliet, 1896, with *C. alatum* as type species. However, a further examination of the extremely useful bulletin by Stiles and Hassall (1908) shows that the genus *Alaria* Schrank, 1788, has as its type, and only, species *A. vulpis*, which is a renaming of *Planaria alata* Goeze, 1782, the fluke which has been called *Hemistomum alatum* since the publication of that name by Diesing, 1850. In default of any earlier nomenclature affecting the status of the generic name *Alaria* Schrank, 1788, or the specific name *alata* of Goeze, 1782, it appears that the fluke from dogs and other carnivores in Europe should be known by the name *Alaria alata* (Goeze, 1782) Hall and Wigdor, 1918. This fluke has been assigned to the family Holostomidae E. Blanchard, 1847, and to the subfamily Hemistominae Brandes, 1890 (name used by Brandes on Plate 40 and overlooked by Stiles and Hassall, who credit this name to Braun, 1893), and the tribe Hemistomeae Brandes, 1890. Since the name of the genus on which the subfamily and tribe names are formed must be changed from *Hemistomum* to *Alaria*, the subfamily and tribe names must be changed to Alariinae and Alarieae.

These flukes belong to the Holostomata, or metastatic trematodes, those which develop without intermediate generations arising by asexual methods. Two larval forms develop and pass through an intermediate host.

The family diagnosis is as follows:

Family HOLOSTOMIDAE E. Blanchard, 1847.

Family diagnosis.—*Holostomata*: Oral sucker terminal or subterminal; ventral sucker usually but slightly developed. Behind the ventral sucker is a peculiar attaching apparatus, varying in shape in different species. The body is commonly divided by a cleft into an anterior and a posterior region. The sex organs are principally in the posterior region and have their common openings at the posterior end in a depression, opening dorsally, called the bursa copulatrix. The oral and ventral suckers, the attaching apparatus, and part or all of the highly developed vitellaria are in the anterior portion. The simple intestinal ceca are without diverticula and extend the entire length of the body. The uterus is but slightly contorted and contains only a small number of relatively large eggs, which develop in water. The adult worms occur in the intestine of mammals, birds and rep-

tiles, rarely in fish and amphibia; the intermediate hosts are mammals, birds, amphibia, fish and mollusks.

Type genus.—*Holostomum* Nitzsch, 1819.

Subfamily ALARIINAE Hall and Wigdor, 1918.

Subfamily diagnosis.—*Holostomidae*: Forms with flattened anterior body portion, of which the lamellar lateral edges are strongly bent ventrally, forming a sort of sac with a long ventral aperture between these lamellar edges. The ventral sucker is often covered by the attaching apparatus and is usually not larger than the oral sucker or the pharynx. (The ventral sucker is lacking in at least one species.) The attaching apparatus is in the form of a compact mass, often covering the greater part of the anterior body. The apertures of some glands are at the sides of the oral sucker. The genital cone and bursa copulatrix are notably developed only in exceptional cases. The opening of the bursa is constantly dorsal. Parasitic in birds and mammals.

Type genus.—*Alaria* Schranck, 1788.

Tribe ALARIEAE Hall and Wigdor, 1918.

Tribe diagnosis.—*Alariinae*: With the characters of the subfamily.

Type genus.—*Alaria* Schranck, 1788.

Genus *Alaria* Schranck, 1788.

Generic diagnosis.—*Alarieae*: Posterior portion of the body approximately cylindrical; anterior portion flattened and with its lateral borders curving toward the ventral surface. The ventral sucker is usually larger than the oral sucker. The attaching apparatus is a compact structure which often covers the greater portion of the ventral surface of the anterior part of the body, and may entirely or partly cover the ventral sucker.

Type species.—*Alaria vulpis* Schranck, 1788 (= *Planaria alata* Goeze, 1782, renamed, = *Alaria alata* (Goeze, 1782)).

The fluke which we found in 4 dogs (1.33 per cent) of the series of 300 dogs is the larger of our two species and is most closely related to *Alaria alata*, the intestinal fluke from dogs and other carnivores in Europe. We propose for this species the name *Alaria americana*. The smaller of our two flukes, which we found in 3 of our 300 dogs (1.0 per cent), we propose to call *Alaria michiganensis*. The three species may be differentiated as follows:

KEY TO SPECIES OF ALARIA FROM THE DOG.

1. No projecting structures at each side of the oral sucker; right testis bilobed, left testis irregular in outline but integral. *Alaria michiganensis*.
Projecting structures at each side of oral sucker; both testes bilobed....2
2. Attaching apparatus covers the posterior portion of the ventral sucker; the field of the vitellaria extends to the ventral sucker; the ventral sucker slightly larger than the oral sucker....*Alaria americana*
Attaching apparatus distinctly posterior to, and not touching or covering, the ventral sucker; the field of the vitellaria in the median line all posterior to the anterior end of the attaching apparatus and to the oral sucker; the oral sucker larger than the ventral sucker*Alaria alata*.

Part of the above key is based on Brandes' (1890) figures and depends for its accuracy on the accuracy of those figures and of our interpretation of them.

More extended descriptions of these species are as follows:

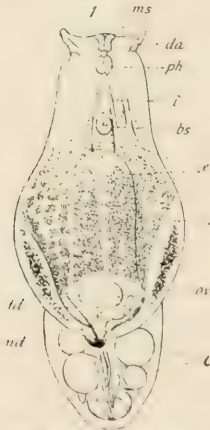


Figure 1. *Alaria alata*. Ventral view. *ms*, oral sucker; *da*, site of gland aperture; *ph*, pharynx; *i*, intestine; *bs*, ventral sucker; *e*, excretory system; *z*, vitellaria; *ov*, ovary; *td*, transverse vitelline duct; *ud*, unpaired vitelline duct; *t*, testes. Magnified. After Brandes (1890).

Species *Alaria alata* (Goeze, 1782) Hall and Wigdor, 1918.

Specific diagnosis.—*Alaria*: Flukes 3 to 6 mm. long (Fig. 1). The posterior body much shorter than the anterior. The oral sucker and pharynx quite distinct and each of them larger than the ventral sucker. Some distance posterior of the ventral sucker

is the attaching apparatus, a high structure, notched anteriorly, according to Brandes' figures, and with prominent lateral margins. The greater part of the vitellaria are contained in the attaching apparatus. In the median line there is a row of apparent cavities, which are actually interruptions in the vitellaria and which are bounded by the dorso-ventral anastomoses of the excretory system. (The cut for Fig. 1 has been tooled in a way that has effaced the representation of these somewhat.) On each

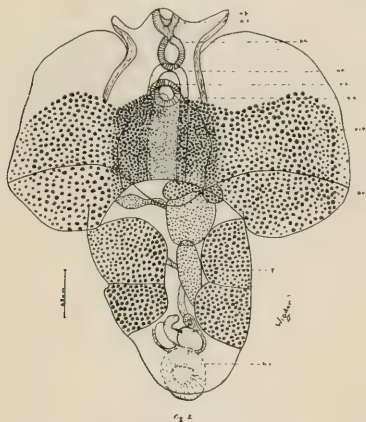


Figure 2. *Alaria americana*. Ventral view. a. p., anterior projection; o. s., oral sucker; ph., pharynx; int., intestine; v. s., ventral sucker; a. a., attaching apparatus; vit., vitellaria; ov., ovary; t., testes; b. c., bursa copulatrix.

side of the oral sucker is a crescentic projection and in these are located the apertures of glands. There is a large bilobed testis on each side of the posterior body. The ovary gives the appearance of being in the anterior body portion, owing to the fact that the lateral lamellae of the flattened anterior portion unite on the ventral surface far back over the cylindrical posterior portion of the body, with the entire ovary anterior of the line of union, according to the figure given by Brandes. The uterus and vas deferens open in the middle of a small genital cone. The bursa

copulatrix opens dorsally, as in other species of this genus, and is relatively insignificant.

HOSTS.—*Canis familiaris*, *Canis vulpes*, *Canis lupus*, *Canis logopus*, *Thoas cancrivorus*, and *Megalotis cerdo*.

LOCATION.—Intestines and, occasionally, stomach.

LOCALITY.—Europe. (Natterer is said to have collected it from *Thoas cancrivorus*, which suggests that the fluke was collected in South America.)

Species *Alaria americana* Hall and Wigdor, 1918.

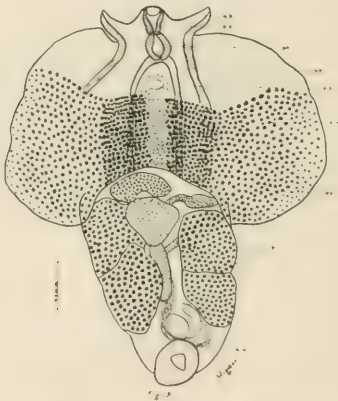


Figure 3. *Alaria americana*. Dorsal view. Lettering as in Figure 2.

SPECIFIC DIAGNOSIS. —*Alaria*: Mounted specimens less than 3 mm. long (Figs. 2 and 5); live specimens appear to be between 4 and 5 mm. long. The posterior portion of the body appears to be shorter than the anterior, but owing to the contractility of the animal, the two parts may appear to be of practically the same length in some specimens. A transverse wrinkling of the cuticle of the equatorial region of the posterior body seems to be common. The oral sucker and pharynx are quite distinct, but their transverse diameters are less than that of the ventral sucker, contrary to the condition in *Alaria alata*. The ventral sucker is relatively well forward, less than its own diameter from the angle

formed by the intestinal ceca, whereas the sucker in *A. alata* appears to be placed a distance distinctly greater than its own diameter behind this angle. The attaching apparatus is similar to that in *A. alata*, but the anterior end is smoothly rounded and does not show the notch which is figured for *A. alata* by Brandes (1890). In the median line of the vitellaria in the attaching apparatus, there are a series of apparent cavities, not presenting sharply defined, occasionally rectangular outlines as in *A. alata*, and usually 3 to 5 in number and not 9 in number as figured for *A. alata* by Brandes. In the median line the vitellaria in flattened specimens extend forward to the same transverse plane as the ventral sucker, the attaching apparatus extending slightly forward of the vitellaria and partly covering the ventral sucker. In *A. alata*, the ventral sucker is well forward of the anterior end of

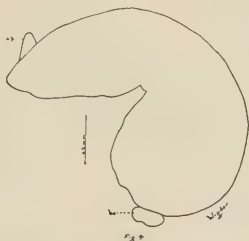


Figure 4. *Alavia americana*. Outline view from side. a. p., anterior projection; b. c., bursa copulatrix.

the attaching apparatus of the vitellaria in the median line. Specimens which have curled up, apparently in response to some irritant stimulus, show the attaching apparatus shoved forward till its anterior end is in the vicinity of the oral sucker, the lateral lamellae of the anterior body being folded over toward the mid-ventral line, and the posterior body being bent back in a way that tends to bring its dorsal portion in contact with the dorsal portion of the anterior body. On each side of the oral sucker are crescentic projections as in *A. alata*, presumably bearing the ducts of glands as the similar structures in *A. alata* are said to do. There is a large bilobed testis on each side of the posterior body. The ovary appears to lie partly anterior to and partly posterior

to the line of union of the lateral lamellar margins of the anterior body. The bursa copulatrix is less than twice the diameter of the ventral sucker, whereas in *A. alata* it is about three times the diameter of the ventral sucker, according to Brandes' figures. The eggs in the uterus are $90\ \mu$ to $120\ \mu$ by $80\ \mu$ to $86\ \mu$ in diameter. Eggs from the feces measured $106\ \mu$ to $134\ \mu$ by $64\ \mu$ to $80\ \mu$ (Fig. 5). Other details of the reproductive system and other systems were not determined, as this study was only incidental to other investigations which would not permit of taking time for the work of making and mounting sections.

HOST.—*Canis familiaris*.

LOCATION.—Small intestine.

LOCALITY.—Detroit, Michigan.

The largest number of *A. americana* found in one animal was 91 (in dog No. 281).

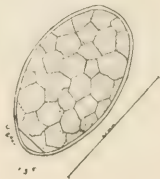


Figure 5. *Alaria americana*. Egg from feces.

Species *Alaria michiganensis* Hall and Wigdor, 1918.

SPECIFIC DIAGNOSIS.—*Alaria*: Flukes 1.8 to 1.91 mm. long when mounted (Figs. 6 and 7). Posterior portion of body longer or shorter than the anterior portion, according to the state of contraction. The anterior portion of the body appears to be covered with minute, posteriorly-directed spines. Well developed oral sucker and pharynx. Oral and ventral suckers of approximately the same size, sometimes one and sometimes the other the larger. The attaching apparatus is usually immediately posterior of the ventral sucker in flattened specimens and has no notch anteriorly. Occasionally it is considerably posterior of the ventral sucker and in a number of mounted specimens we are unable to detect an attaching apparatus. In the median line the vitellaria extend anterior of the ventral sucker to a point between

the ventral sucker and the posterior end of the pharynx. There are no such apparent cavities in the median field of the vitellaria as there are in *A. alata* and *A. americana*. There are no crescentic projections on the sides of the oral sucker. The right testis is bilobed and lies transversely across the posterior body, extending across the median line in such a way that one lobe lies on the right side and one lobe lies on the left side of the worm posterior to the left testis. What appears to be the cirrus can be distinguished on the left side, connecting with the bursa copulatrix. The ovary lies somewhat to the left, instead of median, and is entirely posterior of the line of union of the lateral lamellar margins of the anterior part of the body. The transverse vitelline

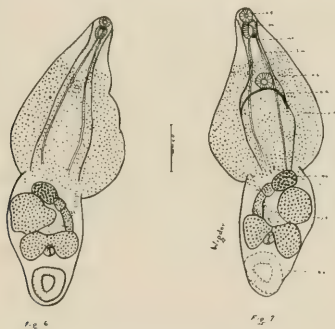


Figure 6. *Alaria michiganensis*. Dorsal view.

Figure 7. *Alaria michiganensis*. Ventral view. Lettering as in Figure 2.

duct crosses to the right side near the union of the anterior and posterior body and the main vitelline duct extends along the right side of the posterior portion of the body and apparently crosses ventrad of the right testis to the left side, forming a dilatation in the median line. The bursa copulatrix is more than twice the diameter of the suckers. The eggs in the uterus are $80\ \mu$ to $104\ \mu$ by $76\ \mu$ to $80\ \mu$ in diameter.

HOST.—*Canis familiaris*.

LOCATION.—Small intestine.

LOCALITY.—Detroit, Mich.

The largest number of *A. michiganensis* found in one animal was 80 (in dog No. 195).

The resemblance between *Alaria alata*, the form described from Europe, and the species we have designated as *A. americana* is very considerable and the possibility of their being the same species should be given consideration. We find certain differences on comparison: Although the measurements overlap, *A. alata* appears to be larger as regards average size and maximum size. In *A. americana* the vitellaria in the median line and the anterior border of the attaching apparatus both lie in the transverse field determined by the antero-posterior diameter of the ventral sucker, whereas in *A. alata* the ventral sucker lies anterior of the forward end of the attaching apparatus, which in turn seems to extend well forward of the anterior limits of the vitellaria in the median line. This difference, the absence of a notch in the anterior margin of the attaching apparatus, and a few other differences in relative sizes of suckers, etc., make it unwise to claim the identity of the European and American form. In this matter we follow the advice of Dr. Ch. Wardell Stiles, who considers it better to take a chance on making a new species for an old one, where there is a doubt, and assuming that an error will be pointed out and the new name assigned its proper status as a synonym, than to record such a doubtful finding under the name of an old species with which it may easily be long confused and from which it will be very difficult to separate it if there is an error.

The measurements of mounted specimens of the two American species of flukes are given in the following table, nothing of the sort, except the body length of 3 to 6 mm., being known to us for *A. alata*:

Structure	<i>A. americana</i>		<i>A. michiganensis</i>	
Entire body	1.16	mm. to 2.32 mm.	1.80	mm. to 1.91 mm.
Anterior body	0.69	mm. to 1.07 mm.	0.80	mm. to 1.17 mm.
	0.71	mm. to 1.96 mm.	0.85	mm. to 0.94 mm.
Posterior body	0.48	mm. to 1.25 mm.	0.72	mm. to 1.11 mm.
	0.65	mm. to 0.95 mm.	0.85	mm. to 0.92 mm.
Oral sucker	0.090	mm. to 0.137 mm.	0.086	mm. to 0.167 mm.
Pharynx	0.120	mm. to 0.196 mm.	0.142	mm. to 0.162 mm.
	0.080	mm. to 0.137 mm.	0.118	mm. to 0.127 mm.
Ventral sucker	0.070	mm. to 0.156 mm.	0.090	mm. to 0.176 mm.
Reproductive organs	0.114	mm. to 0.245 mm.	0.088	mm. to 0.236 mm.

The flukes reported from the dog outside of North America include the following: *Opisthorchis felinus*, *Opisthorchis*

caninus, *Clonorchis endemicus*, *Metorchis albidus* (?), *Pseudamphistomum truncatum*, *Ascocotyle minuta*, *Ascocotyle italica*, *Loossia romanica*, *Heterophyes heterophyes*, *Heterophyes aequalis*, *Heterophyes dispar*, *Isthmiophora melis* (?), *Echinochasmus perfoliatus*, *Dicrocoelium dendriticum*, *Schistosomum japonicum*, *Yokagawa yokagawa* and *Hemistomum alatum*.

In passing it is of interest to note that Stiles and Hassall (1894) report the presence of *Hemistomum alatum* in the collection of the U. S. Bureau of Animal Industry at Washington, D. C., but the material is European, not American. The specimens are from Rudolphi's collection and probably have an historical interest far greater than their scientific value for study after preservation for a century; no effort was made to examine this material for comparison.

BIBLIOGRAPHY.

- BRANDES, GUSTAV. 1890. Die Familie der Holostomiden. *Zool. Jahrb., Abt. f. Syst.*, v. 5 (4), 24, Dec., pp. 549-604, pls. 39-41.
- RAILLIET, ALCIDE. 1896. Quelques rectifications à la nomenclature des parasites. *Rec. de Méd. Vét.*, v. 73, 8 s., v. 3 (5), 15 mars, pp. 157-161.
- STILES, CH. WARDELL, and ALBERT HASSALL. 1894. A preliminary catalogue of the parasites contained in the collections of the United States Bureau of Animal Industry, United States Army Medical Museum, Biological Department of the University of Pennsylvania (Coll. Leidy) and in Coll. Stiles and Coll. Hassall. *Vet. Mag., Phila.*, v. 1 (4), Apr., pp. 245-253; (5), May, pp. 331-354.
1908. Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. *Hyg. Lab. Bull.* (37), 401 pp.
- WARD, HENRY B., and HIRSCH, EDWIN F. 1915. The species of *Paragonimus* and their differentiation. *Ann. Trop. Med. & Parasitol.*, v. 9 (1), Mar., pp. 109-162, pls. 7-11.

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LESIONS DUE TO AGENTS USED IN KILLING EXPERIMENT DOGS IN ANTHELMINTIC INVESTIGATIONS.*

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The investigation of anthelmintics involved in a general way two problems: a consideration of the actual anthelmintic activity displayed in the removal of worms and a consideration of the transient and permanent effects of the anthelmintic on the host; in other words, the effect of the anthelmintic on the parasite and on the host. The anthelmintic value or efficacy of a treatment is ascertained rather accurately by a consideration of the number of worms removed within a reasonable time and the number remaining after that time, the number passed being found by an examination of the feces for the time involved and the number left being found by postmortem examination. This is the experimental laboratory method. Less accurately, the efficacy may be ascertained by a comparison of the number of worms removed by a first treatment with the number removed by a second treatment or other subsequent treatments, and this is the method used in medical practice. The immediate and remote effects on the host may be ascertained by clinical observation, by the more exact methods of the physiological and pathological laboratory, and by postmortem examination. The information available by postmortem examination is unfortunately seldom available in medical practice, but in experimental work all these methods may be employed. The study of the effect of the treatment on the host is highly important, as anthelmintic treatment is often very severe,

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commonly more or less of a shock, and potent anthelmintics are nearly always more or less toxic substances, supposed to be comparatively insoluble, as a rule, but actually rather soluble, which may initiate a series of pathological conditions beginning with gastro-intestinal irritation and inflammation and terminating in death.

DIFFERENTIATION OF LESIONS.

In examining an experiment animal for lesions due to anthelmintic treatment, it is imperative, in the interests of the best work, that one undertake to differentiate the lesions due to the treatment from lesions due to disease, accident, or the agent used in killing the experiment animal, and the present paper is primarily a consideration of the lesions to be expected in connection with a limited number of methods of killing animals, the animals in this case being dogs. This work is based on some of the findings in a study of 300 cases.

The great majority of our experiment dogs were killed in one of three ways: (1) by chloroform inhalation in a box; (2) by intraperitoneal injections of alcoholic solutions of chlore-tone (trichlorotertiarybutyl alcohol, $C_4H_7OCl_3$) in 40 per cent solution at the rate of 1 mil per kilo, as recommended by Rowe (1916)¹, the animal being opened under profound anesthesia; (3) by shooting the animal through the brain with a .22 caliber revolver with a 1.5-inch barrel. In shooting, the short cartridges are apparently as effective as the long, usually, and it makes no difference that we can detect, so far as the lesions are concerned, whether the animal is shot in the frontal region where the cross-lines from the eyes to the opposite ears intersect, or in the parietotemporal region, or in the occipital region.

Certain types of visceral lesions found to result from the employment of these methods in killing dogs are as follows:

Chloroform. Dogs killed by chloroform inhalation very commonly exhibit a hyperemic condition of the mucosa or submucosa of the duodenum or jejunum or, usually, of both. The word *hyperemic* is used here loosely to designate hyperemia, congestion, and inflammation, associated at times with hemorrhagic

¹Rowe, I. W. 1916. Trichlorotertiarybutyl alcohol anesthesia. *J. Pharm. & Exp. Therap.*, Vol. 9 (2), Nov., pp. 107-112.

conditions. In routine postmortem examinations, lesions are sometimes so sharply defined in their nature that macroscopic examination is sufficient to enable one to designate the lesion fairly accurately, but there are borderline cases and vague cases that must be grouped under some general term in a consideration of this sort. This hyperemic condition in dogs killed by chloroform inhalation is of considerable interest in experiments on anthelmintics, since anthelmintics are commonly gastro-intestinal irritants and their irritant effect must be judged largely by the hyperemic condition of the duodenum and jejunum.

Chloretone. Dogs killed by opening under anesthesia due to preliminary intraperitoneal injections of chloretone very commonly exhibit a highly congested spleen, the spleen being occasionally three times as large as normal. Such spleens present a peculiar smooth, tense, bluish capsule as a result of the high pressure of the contained blood. On cutting the splenic vessels or incising the spleen, there is a very rapid and considerable diminution in the size of the spleen, but it rapidly returns to practically normal size. However, it sometimes happens that blood-cysts, what might be loosely termed "blood-blisters," spaces filled with blood, sometimes rather large (1 cm. in diameter), form in the splenic pulp or just under the capsule. This lesion has little significance, as it could not result from the use of any anthelmintic of which I am aware, but it is of interest in that it does not appear to have been known, even by persons familiar with chloretone and habitually using it, that this lesion might result from intraperitoneal injections of the strength noted. Such injections would furnish a convenient method of producing splenic lesions for pathology classes and for certain studies on the spleen.

Shooting. Dogs killed by shooting through the brain commonly exhibit extravasations in the heart muscle and the lung tissue. These extravasations may be petechial or ecchymotic, or may be extensive dark blotches. They may be visible on the inside and outside of the heart and through the muscles wherever incised, or may be limited in location, and may be visible under the pulmonary pleura, through the lung tissue and in the bronchi, especially in the terminal portions, or may be limited in extent.

FINDINGS IN KILLED ANIMALS.

The extent to which these lesions are characteristic of the deaths with which I associate them may be judged from the extent to which they are present and readily visible in animals killed by these three methods, and a consideration of findings for these animals is given next.

Chloroform. Chloroform was used to kill 102 dogs, one of these dogs having been given a preliminary dose of chloretone intraperitoneally. The characteristic hyperemia of the duodenum was present in 69 cases (69 per cent) and of the jejunum in 64 cases (64 per cent). From each of these should be deducted 2 cases, where dangerous doses of anthelmintics, less than the lethal dose but in excess of the safe therapeutic dose, and therefore very apt to occasion hyperemia, had been given, making 67 cases (67 per cent) with hyperemia of the duodenum and 62 cases (62 per cent) with hyperemia of the jejunum, in cases where only therapeutic doses of anthelmintics or even smaller doses, were given. Comparing these figures with those for chloretone, we find that in 36 animals killed after chloretone injections intraperitoneally, 15 showed hyperemia of the duodenal mucosa and 18 showed hyperemia of the jejunal mucosa. From these figures should be subtracted 2 cases where a lethal and a dangerous dose, respectively, were given, making 13 cases (36 per cent) of hyperemia of the duodenum and 16 cases (44 per cent) of hyperemia of the jejunum. Comparing the foregoing figures with those for shooting, we find that in 116 animals killed by shooting, 37 showed hyperemia of the duodenum and 35 showed hyperemia of the jejunum. From these figures for duodenal hyperemia should be subtracted 4 cases of lethal and 4 of dangerous doses, and from the figures for jejunal hyperemia should be subtracted 8 cases of lethal and 5 of dangerous doses, making 29 cases (25 per cent) of hyperemia of the duodenum and 22 cases (19 per cent) of hyperemia of the jejunum.

My attention was first called to the production of hyperemia of the upper portion of the small intestine, following chloroform inhalation, by Dr. Jacob Traum some years ago at a time when he and I were both working in the United States Bureau of Animal Industry. The dilatation of the abdominal vessels under

chloroform was noted years ago by Peck, but it is perhaps not generally known and appreciated.

The explanation for the production of intestinal hyperemia by chloroform is perhaps the following: It may be due in part to swallowing mucus with its chloroform content during anesthesia, the chloroform exerting its irritant effects directly on the intestinal mucosa; and due in part to the fact that chloroform acts as a depressant to the heart and to the vasomotor centers, so that with the resulting fall in blood-pressure the blood tends to collect in the abdominal vessels, perhaps due to the fact that these relaxed vessels have less surrounding pressure on them than the vessels of the skin and voluntary musculature. It appears likely that chloretone, also, may narcotize or anesthetize the vasomotor nerves of the digestive tract, thus producing the hyperemia which occurs oftener in dogs killed after chloretone than in dogs killed by shooting.

The occurrence of this intestinal hyperemia in practically two animals out of every three when killed by chloroform, makes this method of killing experiment animals unsatisfactory for experiment work where the irritant effect of anthelmintics is to be considered.

Chloretone. Chloretone was used in killing 36 dogs. A congestion of the spleen was noted in 21 cases (58 per cent), and in over half of these cases the spleen was from 50 per cent larger than normal to three times as large as normal. In comparison, a congested spleen was found in dogs killed with chloroform in only 6 cases, one of these in a dog previously given chloretone, leaving 5 cases (5 per cent). In dogs killed by shooting, a congested spleen was found in 3 cases (2.6 per cent). In no case where dogs were killed with chloroform or by shooting was a spleen found showing the enormous enlargement found in animals given chloretone intraperitoneally.

The explanation for this congested spleen probably is that the vasomotor nerve control is narcotized or anesthetized, thereby causing an inhibition of the vasoconstrictor nerve control and allowing vascular dilatation and distention.

Shooting. Shooting was employed in killing 116 dogs. Extravasations of the heart were found in 93 (80 per cent) and of the lungs in 78 (67 per cent), and a more careful examination,

with a "shredding" of the heart and lungs, or a microscopic examination, would probably have shown this condition in more animals, though probably not in all of them. In comparison, hemorrhages of the heart were observed in no instances in dogs killed with chloretone or chloroform, and hemorrhages of the lungs were observed only in 2 cases (6 per cent) in dogs killed with chloretone and in 5 cases (5 per cent) in dogs killed with chloroform.

The explanation for these lesions is presumably this: The shot occasions sufficient shock to the brain to destroy the cardio-inhibitory center, the vague inhibition control of the heart is removed, and with this brake off, the heart, under the influence of its accelerator control, immediately races to a speed of 200 to 225 beats a minute, respiration simultaneously ceasing. Under the back pressure of the blood, especially in the pulmonary circulation, the pound of the heart is sufficient to cause these extravasations and, at the other end of the line, the pressure in the pulmonary circulation drives the blood out into the lung tissue. The lungs are almost always distended and remain so after the thorax is opened, instead of collapsing. In one animal, the lobes on one side were distended and showed extravasations, while the other lobes were collapsed and free from extravasations. Extravasations appear to be more numerous and extensive in the left lobes. The ventricles, pumping against the pressure of the systemic and pulmonary circulation, suffer more than the auricles, and the right side of the heart, pumping against the pulmonary pressure, suffers more than the left side. Lesions are visible on the inside of the heart a little oftener than on the outside. Extravasations were visible as follows: In the right ventricle, 64 times; on the right ventricle, 53 times; on the left ventricle, 53 times; in the left ventricle, 45 times; in the right auricle, 31 times; in the left auricle, 27 times; on the right auricle, 23 times; and on the left auricle, 19 times.

Feeble animals are apt to be free from extravasations of the heart and lungs, and it is possible that in the case of these dogs, and some others, the heart is stopped as a result of shock affecting the accelerator control of the heart or as a result of a weak heart stopping under the overload put on it by the back pressure in the lungs.

These lesions do not appear to be generally known. On examination of La Garde's (1916)¹ treatise on gunshot wounds in man, I find no mention of the immediate effect of gunshot wounds of the head on the heart action, and I have not yet been able to get the data on this point in regard to man. The lesions might be mistaken for hemorrhagic septicemia, for those resulting from asphyxia or, to some extent, for those from strychnine poisoning. We found pulmonary hemorrhage in all cases where four dogs were killed with strychnine. It might be urged that a bullet wound in the head is the best evidence that an animal was shot, but occasionally this lesion is hardly discoverable, a small hole with no bleeding and concealed by long hair being easily overlooked.

There are two fields in which lesions of this sort might be looked for in animals. One is the abattoir, where beeves are killed by trauma, "knocking" on the head with a sledge hammer, followed by bleeding (cutting the throat), and the other is the hunting field where game is shot, occasionally through the head or neck, a condition practically identical with that used in our killing of experiment dogs. In regard to the first, I am informed by Dr. E. P. Schaffter, the Bureau of Animal Industry inspector-in-charge at Detroit, that they occasionally find extravasation in beeves, the lesions occurring at times in the muscles of the loins. As a rule they are not present. Apparently the stunning blow on the head is not usually sufficient to destroy the functioning of the cardio-inhibitory center. These lesions are not found in sheep or hogs, which are not stunned. In regard to the second field, hunting, Dr. Clifford E. Parker tells me that he has seen extravasations of the heart or lungs in the deer, fox, and raccoon after being shot in the head or neck, and has seen one raccoon carcass thrown away on account of these lesions.

In passing, it may be said that a dog shot in the right way collapses and stiffens, and then very soon relaxes and begins to wag its tail. The oculopalpebral reflexes are abolished immediately. The frontal shot causes profuse bleeding as a rule, while the parietotemporal or occipital shots may or may not be followed by bleeding. The aperture is sometimes sealed against

¹La Garde, Col. Lewis A. 1916. Gunshot injuries. How they are inflicted. Their complications and treatment. N. Y., 2 ed., 457 pp., 160 figs.

bleeding by a piece of torn brain tissue. While any of these shots will usually kill a dog, I have had bullets flatten and split in the region of the parietal crest and the nuchal crest, and this observation and others lead me to think that with a small calibre gun, the best shot is one carefully centered on the intersection of the cross-line from the eyes to the opposite ears and with the gun pointed back toward the cerebellar region.

Dogs which are run over by automobiles and survive, not infrequently show a damaged spleen, repaired with omental adhesions, as the sole evident lesion. There were 6 such cases in our series of 300, or 2 per cent.

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**SPECIFIC INFECTIOUS DISEASES OF UNKNOWN
ETIOLOGY, WITH SPECIAL REFERENCE TO
ULTRAVISIBLE VIRUSES.***

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The theory of *contagium animatum* originated long before the invention of the microscope. The development of the microscope, culture methods, staining reactions, animal experimentation, and serum tests has led to the demonstration of the specific causative organisms involved in many of the diseases of men and animals. From the study of the microscopical forms present in many pathogenic processes has been evolved the new science of bacteriology, or microbiology. The science of microbiology, which at first was confined to the study of pathogenic organisms and fermentation processes, now includes the vast expanse in which are involved the investigation, correlation, and teaching of facts relating to dairy, soil, and household bacteriology, pathology, hygiene, sanitation, and preventive medicine.

In the excellent words of Rettger,¹ "Bacteriology is a child of many adoptions, ever precocious, but not yet fully mature. Born with a definite mission to serve and to save, it has recreated pathology, given inspiration and new life to botany and zoology, contributed generously of its substance to agriculture and home economics, and built itself as the framework around which modern hygiene and preventive medicine have been built."

We are inclined to regard the study of pathogenic microorganisms as fairly well completed and the science of bacteriology as quite conclusively established in so far as it relates to the study of specific infectious diseases. A most conspicuous break, however, has occurred in the development of the study of microbiology, which relates to a group of specific infectious diseases

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of unknown etiology consisting of nearly forty diseases. This break has been bridged over, in some degree, by the results of the work of many investigators, who have demonstrated that the majority of diseases of this group are capable of being produced by the filtrate of diseased tissues.

Loeffler and Frosch² in 1898 first noted the phenomenon of virus filtration in connection with the study of the virus of foot-and-mouth disease. They found that the serum from infected cattle, after being passed through fine porcelain filters which would not allow the passage of ordinary bacteria, still retained the specific living agents which act as the cause of the disease.

It is interesting to note the manner in which this discovery was made. Loeffler³ conceived the idea that, by passing the exudate from the vesicular lesions of foot-and-mouth disease through the Berkefeld filter, the more active, infective portions of the virus might be retained by the filter, thus rendering the filtrate free from infective properties, but, at the same time, capable of producing protection against the disease when injected into healthy animals. Control experiments proved that no known bacteria could pass through the filters which were employed, and therefore it was concluded that the virus of foot-and-mouth disease consists of some living agent capable of passing through the fine pores of bacteria-proof filters.

That the filterable material is not a strong toxic chemical substance, but does represent virulent living infection in some form, can be shown by transmission of the virus through several generations of the host, and by the addition of germicidal agents to the filtered virus.

In the former experiment a small portion of the filtered virus is injected into a healthy animal. From this animal, after the appearance of symptoms, the virus is collected and filtered, and with it a second animal is inoculated. When carried through a successive series of animals, it becomes impossible to estimate the dilution of the original material, and hence it must be concluded that the virus is not merely an inert toxic chemical substance. The addition of germicidal solutions renders the virus powerless to produce disease.

Since the pioneer work by Loeffler and Frosch, many investigators have contributed to the study of ultravisible viruses. The

results of this work have demonstrated that there are nearly forty specific diseases which belong to this group as shown in the following table:

LIST OF SPECIFIC INFECTIOUS DISEASES OF MAN AND ANIMALS, THE CAUSATIVE FACTORS OF WHICH ARE CLASSIFIED AS FILTERABLE AND ULTRAVISIBLE VIRUSES.

NAME OF DISEASE.	ORIGINAL INVESTIGATOR.	YEAR
1. Foot-and-mouth disease.....	² Loeffler and Frosch.....	1898
2. Mosaic disease of tobacco.....	⁴ Beijerinck.....	1899
3. Pleuropneumonia of cattle.....	⁵ Nocard and Rous.....	1899
4. African horse sickness.....	⁸ MacFadyen.....	1899
5. Fowl pest.....	⁷ Centanni and Saporuzzi.....	1901
6. Yellow fever.....	⁸ Reed and Carroll.....	1901
7. Fowl diphtheria.....	⁹ Marx and Sticker.....	1902
8. Chickenpox.....	¹⁰ Juliusberg.....	1902
9. Pigeonpox.....	¹⁰ Juliusberg.....	1902
10. Sheepox.....	¹¹ Borrel.....	1902
11. Cattle plague.....	¹² Nicolle and Adil-Bey.....	1902
12. Rabies.....	¹³ Remlinger.....	1903
13. Hog cholera.....	¹⁴ Dorset, Bolton, and McBryde.....	1904
14. Infectious agalactia of sheep.....	¹⁵ Celli and DeBlasi.....	1904
15. Catarrhal fever of sheep.....	¹⁶ Robertson and Theiler.....	1904
16. Molluscum contagiosum.....	¹⁷ Juliusberg.....	1904
17. Cowpox.....	¹⁸ Celli and DeBlasi, ¹⁹ Remlinger.....	1904
18. Dengue fever.....	²⁰ Ashburn and Craig.....	1906
19. Infectious stomatitis papulosa of cattle.....	²² Ostertag and Bugge.....	1908
20. Pappatica fever.....	²³ Doerr.....	1908
21. Smallpox.....	²¹ Casagrandi.....	1906
22. Infectious equine anemia.....	²⁴ Caire and Vallée.....	1908
23. Leukemia of fowls.....	²⁵ Ellerman and Bang.....	1908
24. Trachoma.....	²⁶ Bertarelli and Cecchetto.....	1908
25. Poliomyelitis.....	²⁷ Lentz, ²⁸ Landsteiner and Levaditi, ²⁹ Flexner and Lewis.....	1908
26. Guinea pig epizootic.....	³⁰ Petrie and O'Brien.....	1909
27. Milkpox.....	³¹ Ribas.....	1909
28. Guinea pig paralysis.....	³² Romer.....	1909
29. Measles.....	³³ Goldberger and Anderson.....	1911
30. Scarlet fever.....	³⁴ Bernhardt, ³⁵ Cantacuzene.....	1911
31. Flood fever.....	³⁶ Miyajima.....	1911
32. Rat disease.....	³⁷ Novv and Perkins.....	1911
33. Chicken sarcoma.....	³⁸ Rous and Murphy.....	1911
34. Typhus fever.....	³⁹ Nicolle, Conner, and Conseil.....	1912
35. Silkworm jaundice.....	⁴¹ Prowazek.....	1912
36. Osteochondrosarcoma.....	⁴¹ Rous, Murphy, and Tytler.....	1912
37. Guinea pig plague.....	⁴² DeGaspari and Sangiorgi.....	1913
38. Mumps.....	⁴³ Granata (1908), ⁴⁴ Gordon.....	1914

GENERAL CHARACTERS OF ULTRAVISIBLE VIRUSES.

The infective agents comprising this group are characterized by the following fairly constant features:

1. Great infectiousness.
2. Production of active immunity.
3. Invisibility.
4. Filterability.
5. Noncultivability.
6. Wide geographical distribution.

Infectious Character.—Perhaps the most constant character which distinguishes these viruses, as a group, relates to their marked pathogenicity. They are disseminated with ease and rapidity. Their pathogenic power persists in relatively high dilutions.

The marked infectiousness of these viruses is well illustrated by that of foot-and-mouth disease, the control of which has cost this country several millions of dollars. An outbreak of foot-and-mouth disease presents most serious difficulties to our Department of Agriculture, not because of any unusual high mortality attending the disease, but because of the lightning-like rapidity with which the infection spreads, not only from one community to another, but also from one locality to another far distant.

Invisibility and Noncultivability.—The characterizations of invisibility and incultivability are relative and temporary. One notable and most interesting exception is the virus of pleuropneumonia of cattle. Before this virus was proved to be filterable, Nocard and Rous,⁴⁵ by one of the most brilliant strokes in research, succeeded in cultivating the causative organisms by means of cultures in sterile collodion sacs, which were incubated in the peritoneal cavity of the rabbit. Finally, by means of the most improved lenses, the small organisms, now regarded as the smallest visible microscopical forms, were demonstrated.

For several years the routine diagnosis of rabies has depended on the histological examination of the content of the ganglion cells of the hippocampus major and other portions of the suspected brain tissue for the presence of the cell inclusions first demonstrated by Negri,⁴⁶ and since proved to be characteristic of rabid brain tissue. No proof, however, exists that the Negri bodies, although diagnostic, are of etiological significance.

The virus of acute anterior poliomyelitis has received considerable attention during the last few years. Flexner⁴⁷ and his associates isolated a globoid organism, which they claim is the specific causative agent; at the same time Rosenow⁴⁸ and his followers have conducted extensive studies with cocci isolated from the spinal fluid of poliomyelitis cases, including inoculation into monkeys, serum reactions, and the limited use of experimental antisera—all with promising results.

The streptococcus is generally regarded as closely related to scarlet fever, but no definite proof exists as to its specific properties.

Mallory and Medlar⁴⁹ have reported the presence of *Bacillus scarlatinae* in the tonsillar crypts, trachea, and lungs of scarlet fever patients.

Typhus fever, comparatively recently, has been the subject of investigation by Plotz,⁵⁰ who succeeded in isolating a small anaerobic bacillus which he was able to check up by means of animal inoculations.

Active Immunity.—It is especially interesting and of promising significance to note that, among nearly all of the diseases of this group, one finds that in those individuals who survive the infections there exists an extraordinarily high immunity. Active immunity, relatively permanent in duration, characterizes this group of viruses. Because of this fortunate fact it has been possible to develop methods of artificial immunization against a number of these diseases and, in some instances, specific substances for therapeutic treatment.

By treating naturally immune animals, of susceptible species, with relatively large doses of the virus in question, effective antisera have been developed for the specific treatment of a few of these diseases. Among these may be mentioned the antisera of foot-and-mouth disease, hog cholera, and cattle plague.

For a number of these diseases vaccines are available. Smallpox vaccine, the pioneer biologic preparation, is prepared by passage of the smallpox virus through the bovine species, thus modifying its virulence. The Pasteur treatment for rabies, successful for so many years, consists of the fixed virus, attenuated by drying or dialysis.

FILTRATION EXPERIMENTS.

Variations in Filters.—The simplest filter, with the exception of paper, which of course is not impervious to bacteria, consists of diatomaceous or infusorial earth. This may be arranged in layers, pressure applied, and colloidal material retained in the filter. Berkefeld filters are made in three different grades of porosity—small, medium, and large. The ordinary Berkefeld filter, or filters of this type, should always be tested before use, as imperfections are frequently found. The Mandler filter, man-

ufactured by the Infusorial Products Company, Toledo, O., is recommended as a satisfactory candle. Doubtless the results of many uncontrolled and inaccurate filtration experiments have been reported because of faulty filters. A simple test, described by Ferry,⁵¹ originally reported by Bulloch and Craw,⁵² consists in attaching the filter candle to a compressed air tube, through which the pressure is registered on a gauge. The filter is then immersed in a glass container filled with water, the air pressure applied, and observation made of the air bubbles as they escape through the pores of the filter into the water. For ordinary filtration work the filter candle should maintain 8 to 12 pounds pressure, while the escaping air bubbles should be uniform in size and density over the entire surface of the candle. Imperfections, when found, usually occur near the base or joint of the filter.

Filters of finer degree of porosity are illustrated by the Chamberland and Pukall candles, which are made of porcelain. The virus of foot-and-mouth disease will readily pass through the more porous Berkefeld filters, but, if it is forced through the porcelain filter a few times, the infective agents are retained. The virus of many other infections, such as those of chicken plague and hog cholera, will pass through the finest porcelain, even after repeated passages through the same filter.

A further development in filtration experiments has been brought about by means of the "ultrafilter." The method was first devised by chemists for the purpose of holding back colloid material. It consists in the use of ordinary filter paper, which is thoroughly impregnated with collodion, gelatin, or agar. Bechold has constructed an improved apparatus based on this method. Filter papers, properly impregnated with collodion, are placed on perforated discs so constructed that pressure of known degree may be applied. Fluids pass through such a filter with comparative difficulty. Smallpox virus has been first covered by layers of agar, which retained the infective agents, as no virus was contained in the filtrate. Betegh⁵³ has succeeded in demonstrating that hog cholera virus may be retained by the Bechold filter.

These experiments show that among this group of viruses the infective agents in some instances may be retained by filters of

sufficiently small porosity, thus suggesting that there exist specific ultraviable, pathogenic bodies of different sizes.

Filtration by gravity alone, or with very low pressure, results in the passage of small motile, flexible microorganisms. When relatively strong pressure is used, small particles are rapidly forced into the canaliculi of the filter, thus filling the pores of the filter and blocking further passage.

In conducting filtration experiments, therefore, it is necessary to observe the following points: Variation in filters, absorption of colloidal material, pressure used in filtration, and time of filtration. Finally, in order to prove that the filtration experiment has been accurately and successfully carried out, the experiment should be controlled by cultural and inoculation tests. Cultures made on various artificial media from the filtrate should remain sterile. This proves that no known bacterial forms can be passed through the filter. The inoculation of animals susceptible to the virus, supposed to be contained in the filtrate, should result in the production of the disease in question, involving phenomena typical of the disease, such as proper period of incubation, typical symptoms, death, and characteristic lesions. One of the difficulties involved in the study of filterable viruses depends on the fact that the specific virus in most cases can be recognized only by inoculation of susceptible species of animals. For illustration, hog cholera virus will produce the disease only in swine, the various species of ordinary laboratory animals being entirely insusceptible to hog cholera virus.

Wolbach, Chapman and Stevens⁵⁴ have conducted some interesting filtration experiments from which they conclude that "trypanosomes from cultures and from animal tissues are not filterable through bacteria-proof filters."

Novy and MacNeal⁵⁵ have reported the successful passage of *Spirochæta duttoni* through Berkefeld filters. Wolbach and Binger⁵⁶ have cultivated two filterable spirochetes, *Spirochæta elusa* and *Spirochæta biflexa*, both isolated from water.

ULTRAMICROSCOPY.

No successful work has been done toward the development of microscopical methods which will demonstrate the small organisms assumed to be present in the viruses of this group of diseases. According to Loeffler,³ Abbé and Helmholtz estimate the

limit of microscopical vision as about 0.2 micron. In other words, a microorganism which is larger than 0.2 micron may be seen distinctly by means of a properly constructed and adjusted system of lenses. An organism less than 0.2 micron in diameter may be magnified by means of powerful lenses, but in so doing the distinctness of the image is obliterated, so that no accurate vision of the image can be obtained.

It is, of course, a well-known fact that there is a great variation of size among the ordinary bacterial forms. One of the smallest known pathogenic organisms is the *Bacillus influenzae* of Pfeiffer, which is about one micron in length and four-tenths microns in width. The small *Bacillus murisepticus* is a tiny organism in contrast to the large, square-ended *Bacillus anthracis*. Since there exists such a variation in size and form among bacteria which are well known, it seems quite logical to believe that there are living microorganisms smaller than the influenza bacillus or the bacillus of mouse septicemia, which, under present conditions as to microscopical facilities, are ultravisible. Moreover, it seems reasonable to conclude, from the results of various filtration experiments in which filters of varying degrees of porosity are used, that this group of ultravisible microorganisms may vary among themselves as to size and form.

In attempting to perfect means by which the range of microscopic vision might be developed still further, investigators have attempted to work with light of shorter wave lengths than ordinary light. Ultraviolet rays, whose wave lengths are only one-half as long as ordinary light, have been tried and found to be of no practical advantage.

Among various staining methods which have been tried are those which were submitted by Loeffler and others for the purpose of rendering visible the flagella of bacteria. Such methods, however, appear to be of no avail when applied to the various ultravisible viruses.

The dark ground illumination method, which was developed a few years ago and which has found a place in practically every working bacteriological laboratory, probably represents the most successful practical advance of recent years in perfecting the technique of microscopical observation. By means of the dark ground illuminator the images of small microscopical forms are

reflected by strong light on a dark background, thus affording a means of studying to great advantage small microorganisms in the living condition. Such an apparatus is almost indispensable in studying such forms as spirochetes.

ARTIFICIAL CULTIVATION.

Cultural experiments have been made with practically all of the viruses involved in this group of diseases. In so far as artificial cultivation of a given virus is concerned, the problem depends on the simulation of conditions existing in the host, as related principally to food requirements and thermal conditions. It is for this reason that ordinary bacteria, which thrive on living or dead tissue, prefer artificial culture media in which beef bouillon and peptone form a basis, while, at the same time, soil bacteria flourish on artificial media composed of soil infusions.

Attempts have been made to cultivate the ultravisible viruses on every conceivable kind of culture medium, including fluid and solid media with acid, neutral, and alkaline reactions, subjected both to aerobic and anaerobic conditions, and to various oxygen pressures, with the addition of small particles of tissue, fresh blood, dissolved blood, albuminous materials of various kinds and their cleavage products, different sugars and other combinations which it has been possible to devise.

Mention has already been made of the wonderful work of Nocard and Roux,⁴⁵ who succeeded in growing the virus of cattle pleuropneumonia by means of the collodion sac. This method has been unsuccessfully tried with other filterable viruses. Borrel⁵⁷ succeeded in culturing the virus of sheepox under the skin of the abdomen of sheep, but it was not possible to demonstrate the character of the organism present in the fluid which accumulated in the artificial abdominal pocket. Borrel found that the fluid obtained from such an artificial culture was active in dilutions of from 1:10,000 to 1:20,000. He also showed that healthy sheep could be protected against the disease by the simultaneous use of this virus and antishoop serum in proper amounts.

Marchoux⁵⁸ succeeded in artificially cultivating the etiologic factor of fowl pest on a culture medium consisting of glucose agar, upon which was placed a layer of defibrinated chicken blood. After several days' incubation a second culture was

inoculated from the first; from this a third culture, and so on until ten generations had passed. After ten generations on this artificial culture medium, $\frac{1}{2}$ c.c. of the fluid killed fowls in two days, and Borrel estimated that the dilution of the original virus was of such degree that the fowls which received the killing dose of virus from the culture representing the tenth generation were inoculated with approximately 1 c.c. of the original virus, diluted in a volume of fluid equal to the size of the earth. Thus it was shown that actual multiplication took place in Marchoux' artificial cultures, but the nature of the ultravisible organisms remained undetermined.

The work of Plotz in connection with typhus fever and that of Flexner and Noguchi, as well as the work of Rosenow, of the Mayo Clinic, in studying poliomyelitis, have been mentioned. It is quite possible that the etiologic factors involved in some of these diseases may prove to be organisms which have been entirely overlooked, and which are not ultravisible or noncultivable. Failure to recognize some of these forms may be due entirely to the wrong choice of conditions under which they have been studied, and the absence or neglected observation of various significant clues which might lead to their identity. As an illustration of this point, many will remember the work of Dr. Theobald Smith, and his associates years ago, in attempting to determine the cause of Texas fever in cattle. These workers carried out an extensive series of experiments on the blood of cattle infected with Texas fever, as well as carefully planned isolation experiments for the purpose of determining the mode of transmission of the disease. The latter work resulted in the clear demonstration of the role of the cattle tick as the intermediary host. After this was determined, elaborate work was done on the parasite, including the histological study of many serial sections of the cattle tick, in the hope that the Texas fever organism in certain stages of its life cycle might be found in some of the sections. Finally the outcome of the problem, in so far as the recognition of the organism concerned, looked almost hopeless until one of the workers observed some small, pear-shaped bodies in the red blood corpuscles of cattle suffering from the disease. Comparison of smears of this blood with those made from healthy cow's blood showed the absence of the peculi-

arly shaped bodies in the red blood corpuscles of the blood of normal cattle. Diligent study, with this clew in hand, led to the absolute conclusion that these pear-shaped bodies were protozoans, and represented the specific etiological factor involved in Texas fever.

Strange as it may seem, the slides previously made, consisting of smears from a great number of infected cattle, and the sections of the cattle ticks which had ingested the blood of infected cattle, showed clearly the presence of this organism now recognized as *Piroplasma bigeminum*, the specific protozoan of Texas fever.

OTHER MORE IMPORTANT FILTERABLE VIRUSES RELATED TO THE SPECIFIC INFECTIOUS DISEASES OF MAN.

Under this head, one might consider rabies, smallpox, mumps, acute anterior poliomyelitis, yellow fever, measles, scarlet fever, typhus fever, trachoma, molluscum contagiosum, sand fly or pappataci fever, dengue fever, and foot-and-mouth disease.

RABIES (LYSSA).

Definition.—An acute infectious disease of warm-blooded animals, characterized by acute onset, mental excitement, convulsions, paralytic symptoms, and high mortality.

Etiology.—Filterable virus (Remlinger¹⁸).

Resistance.—The virus is destroyed in half an hour at temperature of 55°-58° C., by gastric juice in 4½ hours, and by bile in a few minutes. It is easily destroyed by ordinary antiseptic solutions. The virus is somewhat resistant to drying, freezing, and putrefaction.

Cell Inclusions.—Negri bodies are specific.

Cultivation.—Moon⁵⁹ has submitted a preliminary report, showing the results of successful cultivation of the virus on an artificial culture medium as demonstrated by inoculation experiments. Poor and Steinhart⁶⁰ have described the results of their study of the virus after obtaining it free from the cells of the host and contaminating organisms. Noguchi⁶¹ reported the observations of multiplying nucleated bodies in artificial culture media inoculated with rabies virus. These nucleated bodies, which Noguchi regards as protozoa, were capable of producing the disease when inoculated into experimental animals.

Laboratory Diagnosis.—Accurate diagnosis is dependent on the observation of Negri bodies in the brain tissue of the animal transmitting the disease. Positive findings of Negri bodies may be verified by the inoculation of rabbits, subdurally, with suspensions of the suspected brain tissue. If positive, "dumb rabies" will be produced in 14 to 18 days.

Transmission.—Propagated chiefly by the dog. The virus is contained

in the nervous system, and is transmitted in some of the secretions, especially the saliva.

Period of Incubation.—Varies; average is six to eight weeks. In some cases it has been less than two weeks and in others one year or more.

Immunity.—Active immunity originally determined by Pasteur, whose vaccine has been used successfully for many years. Passive immunity not established.

SMALLPOX (VARIOLA).

Definition.—"An acute infectious disease characterized by a cutaneous eruption, which passes through the stages of papule, vesicle, pustule, and crust" (Osler).

Etiology.—Filterable virus (Casagrandi, 1908).

Filterability.—Virus passes through Berkefeld filters.

Resistance.—Resists 50 per cent glycerin for eight to ten months, or drying for several weeks. Destroyed at temperature of 58° C. in 15 minutes, and shows weak resistance toward ordinary antiseptic solutions.

Cell Inclusions.—Studied by Guarnier²² (1892) and Councilman, Brinckerhoff, and Tyzzer²³ (1906), and thought by some to be specific protozoa.

Cultivations.—Belin²⁴, Steinhardt, Israel, and Lambert²⁵ (1913) have recorded the results of the incubation of the virus of vaccinia in tissue cultures. A definite multiplication or increase was noted, but no specific bodies were observed.

Laboratory Diagnosis.—Force²⁶ suggests the intradermal inoculation of vaccinia immune rabbits with the vesicular contents, which will produce an allergic reaction.

Transmission.—The virus is readily transmitted by fomites and by individuals who have come in contact with the disease.

Period of Incubation.—Nine to fifteen days.

Immunity. Active immunity originally observed by Jenner. Passive immunity not successful.

MUMPS.

Definition.—"A specific disease characterized by fever and swelling and tenderness of the salivary glands, usually of the parotids, but sometimes of the submaxillary and sublingual glands" (Osler and McCrae).

Etiology. Filterable virus reported by Granata²⁷ (1908) and by Gordon²⁸ (1914), confirmed by Wollstein,²⁹ who succeeded in developing parotitis and orchitis in experimental animals from inoculations of sterile filtrate of infected saliva.

Transmission.—Usually by direct contact.

Period of Incubation.—Average period is about three weeks.

Resistance.—The virus is destroyed at 55° C.

Laboratory Diagnosis.—Eciling³⁰ calls attention to blood changes, including lymphocytosis, which are of value in differentiating mumps from other inflammatory swelling of the parotid and submaxillary glands.

Immunity.—Active immunity is usually conferred by one attack.

ACUTE ANTERIOR POLIOMYELITIS.

(Infantile paralysis, Heine-Medin's disease.)

Definition.—"An acute infection occurring in both epidemic and sporadic forms, characterized anatomically by wide-spread lesions of the nervous system, with special localization in a majority of cases, in the anterior horns of the gray matter in the spinal cord" (Osler).

Etiology.—Filterable virus (Lentz,²⁷ Landsteiner and Levaditi,²⁸ Flexner and Lewis²⁹).

Filterability.—Virus passes through Berkefeld and Chamberland filters.

Resistance.—Resists glycerin one month, drying and freezing for several weeks. Destroyed in half an hour at temperature of 45° C. and by relatively weak antiseptic solutions. Dochez³⁰ has shown that it survives the action of the gastric and intestinal secretions when swallowed.

Cultivation.—Flexner and Noguchi³¹ have successfully cultivated the virus under anaerobic conditions, and have identified the specific micro-organism as consisting of "globoid bodies measuring from 0.15 to 0.3 μ in diameter, arranged in pairs, chains, and masses, according to the conditions of growth and cultivation." This microörganism passes through Berkefeld filters. No attempt has been made to classify it, and it is not known what scale of living organisms these globoid bodies occupy.

Transmission.—Transmitted experimentally through monkeys by injecting the virus as found in the central nervous system. Natural method of transmission is not known.

Period of Incubation.—Five to ten days.

Immunity.—Active immunity has been produced experimentally by the use of graded doses of virus.

Passive immunity, according to the experiments of Flexner and Lewis on monkeys, is possible.

YELLOW FEVER.

Definition.—"A fever of tropical and subtropical countries, characterized by a toxemia of varying intensity, with jaundice, albuminuria, and a marked tendency to hemorrhage, especially from the stomach, causing the 'black vomit'" (Osler).

Etiology.—Filterable virus, Reed and Carroll,³ 1901.

Filterability.—Passes through Berkefeld and Chamberland filter B.

Resistance.—Destroyed in ten minutes at temperature of 55° C. and at 24°-30° C. in 48 hours.

Transmission.—By mosquitoes, *Stegomyia fasciata*. Cannot be transmitted by fomites. The blood of the patient appears to contain the virus for a period of only about three days after disappearance of symptoms.

Period of Incubation.—Forty-one hours to five and one-half days (experimental).

Immunity.—Active immunity usually follows one attack, as shown by evidence submitted by Carter.³²

(MEASLES.)

Definition.—"An acute, highly contagious fever, with specific localization in the upper air passages and in the skin" (Osler).

Etiology. A filterable virus (Goldberger and Anderson⁷⁵). Found in the blood, buccal, and nasal secretions and in the skin. The Rhesus monkey is susceptible to the disease through inoculation with the blood of patient.

Filterability.—Virus passes through Berkefeld filters.

Resistance.—Virus resists drying or freezing for 24 hours. Destroyed by a temperature of 50° C. in 15 minutes.

Laboratory Diagnosis.—Blood examination shows a leucopenia and eosinopenia (Hecker⁷²).

Transmission.—Direct contagion is very common. Transmitted by fomites.

Period of Incubation.—Seven to eighteen days; average incubation period is about 14 days.

Immunity and Mortality. According to Herman,⁷³ 44,680 deaths occurred from measles in the United States from 1900 to 1910. About 30,000 cases are reported annually in New York City, and it is estimated that 95 per cent of the entire population of this country are infected with measles at some period of life.

Infants under 5 months are relatively immune. One attack of measles usually confers immunity for life. Herman suggests the inoculation of children under 5 months old with active filtered virus for protective purposes. Passive immunity not successful.

SCARLET FEVER (SCARLATINA).

Definition.—"An infectious disease characterized by diffuse exanthem and an angina of variable intensity" (Osler).

Etiology.—Little definite knowledge exists as to the nature of the cause. Bernhard⁷⁴ and Cantacuzene⁷⁵ reported the successful infection of monkeys with bacteria-free Berkefeld filtrates.

Resistance.—The virus is quite distinctive in that it is relatively resistant toward ordinary methods of disinfection. The infective agent survives drying for some time.

Cell inclusions.—Mallory⁷⁴ (1904) described protozoan-like inclusions in the skin lesions. Ross⁷⁵ found large cell inclusions within the cytoplasm of the large lymphocytes during the acute febrile period.

Bacteriology.—*Streptococcus pyogenes* is found in the nasal secretions, in the throat, and in the blood of patients. Class⁷⁶ found a diplococcus in the secretions of the throat and later Schultze⁷⁷ and also Ferry⁷⁸ found a diplococcus somewhat similar to the form described by Class. Pryer and Kelly⁷⁹ have isolated a pleomorphic diphtheroid, which varies from a coccus to a rod-shaped form. Mallory and Medlar⁸⁰ have described *Bacillus scarlatinae*, which they regard of etiological significance.

Transmission.—The disease is extremely contagious. The infection is carried by clothing, bedding, furniture; successful disinfection is difficult.

Period of Incubation.—One to seven days, more often 2-4 days.

Immunity.—Active immunity is conferred by one attack.

TYPHUS FEVER.

(Hospital fever, spotted fever, jail fever, camp fever, ship fever.)

Definition.—"An acute infectious disease of unknown origin, highly contagious, characterized by sudden onset, maculated and hemorrhagic rash, marked nervous symptoms, and a cyclical course terminating by crisis" (Osler).

Etiology.—Nicolle⁵⁹ reported the filterability of the European virus. Ricketts and Wilder,⁸⁰ Anderson and Goldberg,⁶¹ Gavino and Girard,⁸² working with the American virus, were not able to confirm Nicolle's results.

Filterability.—Nicolle was able to pass the virus through the coarser Berkefeld filters.

Resistance.—The thermal death point of the virus is 55° C.

Cultivation.—Ricketts and Wilder observed a few small bacilli in the blood of Mexican patients. Plotz⁵⁰ describes a bacillus as the specific cause.

Transmission.—Transmitted by the body louse (*Pediculus vestimentorum*). The virus is present in the blood, especially a few days after the fever has appeared, and the infection is probably present in expired air and on the skin. The infectious agent is carried by fomites.

Period of Incubation.—About 12 days.

Immunity.—Active immunity follows one attack. Slight passive immunity follows the injection of the specific antiserum.

TRACHOMA.

Definition.—"An inflammation, generally of lengthy duration, accompanied by hypertrophy of the conjunctiva and formation of granules, with subsequent cicatricial changes" (May).

Etiology.—There is more or less secretion from the inflamed tissues; the secretion affords a carrier of contagion. The filterable nature of the virus was observed by Bertarelli and Cecchetto²⁸).

Filterability.—The virus passes through the Berkefeld filters.

Resistance.—Not determined.

Cell Inclusions.—Some observations have been made of inclusion bodies in the affected conjunctival tissue cells.

Transmission.—By contact with the secretions. When one eye is involved, frequently transferred to other eye. The disease spreads in schools and public institutions by means of towels, handkerchiefs, etc.

MOLLUSCUM CONTAGIOSUM.

Definition.—"Small, soft, multiple growths of the skin, most frequent on the face, arms, and chest, and in the external genital organs. They are lobulated and contain cells similar to those of the rete malpighii" (Delafeld and Prudden).

Etiology.—Filterable virus described by Juliusberg,¹⁷ found in the degenerated epithelial cells.

Filterability.—According to Juliusberg the virus passes through the Chamberland filter.

Cell Inclusions. Round or ovoid bodies are found within the cells, crowding the nuclei to one side. Some investigators believe these to be protozoa.

Transmission.—By contact through skin abrasions.

PHLEBOTOMUS FEVER.

(Sand fly, three-day or Pappataci fever.)

Definition.—"A fever of two or three days' duration, caused by the bite of the sand fly *Phlebotomus pappataci*." A tropical disease.

Etiology.—Filterable virus described by Doerr.²³ Active immunity follows one attack. Passive immunity not established. The virus present in the blood of the patient.

DENGUE (DENGUE FEVER).

Definition.—"An acute infectious disease of tropical and subtropical regions, characterized by febrile paroxysms, pains in the joints and muscles, an initial erythematous and a terminal polymorphous eruption" (Osler).

Etiology.—Filterable virus described by Ashburn and Craig.²⁰

Filterability. Virus passes through bacteria-proof Berkefeld filters. The virus is present in the blood.

Transmission.—Transmitted by bite of the mosquito, *Culex fatigans*.

Immunity. Active immunity of several years' duration follows one attack. Passive immunity not determined.

FOOT-AND-MOUTH DISEASE.

Definition. An acute disorder, marked by its extreme infectiousness and rapid dissemination, and characterized by fever and the appearance of vesicles in the buccal cavity and around the coronet of the foot.

Etiology. A filterable virus as determined by the pioneer work of Loeffler and Frosch.²

Filterability. The virus passes readily through the ordinary Berkefeld filters, but, if passed many times through the porcelain filters (Chamberlain), it is held back (Loeffler³).

Resistance. The virus is destroyed in 10 minutes at 50° C., drying for 24 hours, freezing for five weeks, and by the addition of the common antiseptics.

Cell Inclusions. Siegel has observed certain cell inclusions to which he ascribed pathological significance.

Transmission. The disease is contagious and is transmitted to man rather rarely, frequently to cattle, sheep, and swine, and sometimes to other domestic animals.

Immunity. Active immunity follows one attack. Passive immunity can be shown by the injection of serum from immune animals.

GROUPING OF ULTRAVISIBLE VIRUSES ACCORDING TO FUNCTIONAL ACTIVITY AND ATTENDING PHENOMENA.

In attempting to classify these diseases we find that there are practically only two features which are common to all—namely, the marked infectivity of the viruses and the production of active immunity conferred by one attack of the disease in question. There are, in addition, several variable features, so different, in fact, that very little assistance may be derived from their use in attempting to classify the group of viruses. These variable features include the following:

1. Production of passive immunity.
2. Methods of transmission.
3. Resistance to chemical agents, drying, and putrefaction.
4. Presence of secondary infection.

In some few instances passive immunity has been demonstrated, as illustrated by the antisera for the treatment of hog cholera and foot-and-mouth disease. With the majority of these viruses, however, the experimental production of passive immunity has not been successful, which would indicate the absence of specific toxin production.

The transmission of this group of diseases, as shown by Wolbach,⁸³ is brought about in various ways, and may be classified under the following heads:

1. Intermediate hosts.
2. Contact.
3. Air borne.
4. Fomites, skin abrasions, and unknown means of dissemination.

Those viruses which are transmitted by biting insects are dengue (*Culex fatigans*), yellow fever (*Stegomyia fasciata*), and African horse sickness (*Anopheles* and *Stegomyia*). Some other viruses are probably transmitted by insects or parasitic organisms which may not act as necessary intermediary hosts.

A few of these viruses require direct contact in order to produce infection. Such consist of infectious agalactia of sheep, infectious stomatitis of cattle, fowl diphtheria, fowl pest, cattle plague, and pleuropneumonia of cattle. Probably many of these viruses may be borne by the air, such as foot-and-mouth disease, scarlet fever, smallpox, and hog cholera.

The majority of the viruses belonging to this group are disseminated in various ways, including fomites and unknown methods of transmission: rabies, chicken sarcoma, and molluscum contagiosum find their entrance by means of abrasions in the skin or tissues.

Sufficient work has not been done to classify successfully the ultravisible viruses according to their relative resistance to chemical agents, drying, and putrefaction. From results obtained thus far it has been shown that there exist great variations, especially in regard to the action against various antiseptic solutions and chemical reagents. Meyer²⁴ gives a very interesting summary of some of the results which have been obtained, and which point to the importance of further carefully planned tests in order to group systematically the viruses according to their resistance toward different chemicals.

Secondary infection is an important factor in connection with most of the diseases which may be classed under those of unknown etiology. As a good illustration of this, mention might be made of the streptococcus in connection with scarlet fever; the pneumococcus, streptococci, and the staphylococci in measles; *bacillus necrophorus*, *bacillus cholerae suis*, and various pyogenic bacteria in hog cholera; and pyogenic cocci in poliomyelitis.

It will be seen that the study of filterable viruses has only begun and offers a wide field for investigation. We are positive of only a few facts in connection with them, the more important of which are that they are ultravisible, as far as it has been possible to determine up to the present time, that they are extremely contagious, that most of them are filterable through the finest porcelain filters, that the viruses are pathogenic even in extreme dilutions, that the production of active immunity is a common characteristic, and that passive immunity production, so far as has been determined, is practically absent.

Some of these diseases may be due to organisms which may readily be seen at certain stages in their life cycle, but which as yet have not been observed because of their possible dissimilarity to other microscopic forms which are better known. Some may be due to protozoan forms, the specific identity of which is difficult to determine; others may be due to spirochetes or some

other forms of microscopic life which are capable of breaking up into small constituents, thus permitting their passage through fine filters, and rendering them ultravisible; others may be due to ordinary bacterial forms which present methods of microscopical technic and artificial cultivation are unable to detect.

No field of research is more inviting than that of the study of the ultravisible viruses. The fundamental study of any specific infectious disease is based on the observation and accurate identification of the specific causative factor. If the specific organism is known and thoroughly studied, it is then usually possible to determine in a scientific way and with accuracy the pathology and proper therapeutic measures. Before attempting to develop preventive and curative measures, the specific causative organism should, if possible, be determined. When this is once recognized, general methods now in use in connection with other diseases may be applied without difficulty, and often with success, in attempting to formulate methods for preventive and curative treatment.

There can be no doubt, therefore, that this field requires more intensive study than it has heretofore received, and that it offers to the research worker the finest opportunity for practical achievement.

REFERENCES.

- ¹Rettger (Jour. Bact., III, 1918, p. 103).
- ²Loeffler and Frosch (Centralbl. f. Bakteriologie, I, Abt., XXIII, 1898, p. 371).
- ³Loeffler (Berl. Tierärz. Wchnschr., XXX, 1914, p. 15).
- ⁴Beijerinck (Centralbl. f. Bakteriologie, II, Abt., V, 1899, p. 22).
- ⁵Nocard and Rous (Ann. de l'Inst. Pasteur, XII, 1898, p. 240).
- ⁶MacFadyean (Jour. Comp. Path. and Ther., XIV, 1901, p. 103).
- ⁷Centanni and Savoruzzi (Centralbl. Bakteriologie, I, Abt., XXI, 1902, p. 183).
- ⁸Reed and Carroll (Compilation, document 822, 61st Cong. 3d session).
- ⁹Marx and Sticker (Deutsch. Med. Wchnschr., XXIX, 1903, p. 79).
- ¹⁰Juliusberg (Deutsch. Med. Wchnschr., XXX, 1904, p. 1576).
- ¹¹Borrel (Ann. de l'Inst. Pasteur, XVI, 1903, p. 123).
- ¹²Nicollé and Adil-Bey (Ann. de l'Inst. Pasteur, XVI, 1902, p. 56).
- ¹³Remlinger (Ann. de l'Inst. Pasteur, IV, 1906, p. 337).
- ¹⁴Dorset, Bolton, and McBryde (Dept. Agr. B. A. I., Bull. 72, 1905).
- ¹⁵Celli and DeBlasi (Clin. Vet., XXVII, 1904, p. 129).
- ¹⁶Robertson and Theiler (Jour. Comp. Path., XVII, 1904, p. 221).
- ¹⁷Juliusberg (Deutsch. Med. Wchnschr., XXXI, 1905, p. 1598).
- ¹⁸Celli and DeBlasi (Riv. Crit. di Clin. Med., XLII, 1903).
- ¹⁹Remlinger (Bull. de l'Inst. Pasteur, IV, 1906, pp. 337, 385).
- ²⁰Ashburn and Craig (Jour. Science, II, 1907, p. 93).
- ²¹Casagrandi (Annali d'Igiene Operin., XVI, 1906, pp. 115, 577; XVII, 1907, p. 551).
- ²²Ostertag and Bugge (Ztschr. f. Infektionskrankheiten d. Haustiere, I, 1906, p. 3).
- ²³Doerr (Berl. Klin. Wchnschr., XLV, 1908, p. 1847).
- ²⁴Carre and Vallée (Compt. rend. Acad. d. sci., CXXXIX, 1904, p. 331).
- ²⁵Ellermann and Bang (Centralbl. Bakteriologie, I, Abt., XLVI, 1908, p. 595).
- ²⁶Bertarelli and Cecchetto (Centralbl. Bakteriologie, I, Abt., XLVII, 1908, p. 432).
- ²⁷Lentz (Ztschr. f. Tyg., LXII, 1909, p. 63).
- ²⁸Landsteiner and Levaditi (Ann. de l'Inst. Pasteur, XXIV, 1910, p. 833).
- ²⁹Flexner and Lewis (Jour. Exper. Med., XII, 1910, p. 227).
- ³⁰Petrie and O'Brien (Jour. Hyg., X, 1910, p. 287).

- ²¹Ribas (Nota Preliminar lida na. Soc. de Med. E. Cir. d. S. Paolo. Enn. Sessao de 5 de Setembro de 1910, S. Paolo, Typagr. Brozie-Rothschild, 1910).
- ²²Römer (Centralbl. Bakteriöl., L, 1911, I, Abt., Beiheft, 50, Ref., p. 30).
- ²³Goldberger and Anderson (Jour. A. M. A., Sept. 16, 1911, p. 791).
- ²⁴Bernhardt (Deutsch. med. Wehnschr., 37, Jahrg. 17, p. 791, April 1, 1911).
- ²⁵Cantacuzene (Compt. rend. Soc. de biol., LXX, 1911, p. 403).
- ²⁶Miyajima (Centralbl. Bakteriöl., I, Abt., 1911, I. Ref. Beiheft, 34-36).
- ²⁷Novy and Perkins (Read at 11th meeting Am. Assn. Path. and Bact., rep. Mich. Acad. Sci., 1912 and 1913).
- ²⁸Rous and Murphy (Jour. Exper. Med., XIII, 1911, p. 397).
- ²⁹Nicolle, Connor, and Conseil (Ann. de l'Inst. Pasteur, XXV, 1911, 97).
- ³⁰Prowazek (Centralbl. Bakteriöl., I, Abt., LXXVII, Orig. 1912, p. 268).
- ³¹Rous, Murphy, and Tytler (Jour. Am. Med. Assn., LIX, 1912, p. 1793).
- ³²DeGaspari and Sangiorgi (Riv. d'ig. E. San. Pubbl. Torino., XXIV, 1913, p. 638).
- ³³Granata (Med. Ital., VI, 1908, p. 647).
- ³⁴Gordon (Rep. to Local Gov. Board on P. H. Med. Subjects, London, 1914, No. 96).
- ³⁵Nocard and Rous (Ann. de l'Inst. Pasteur, XII, 1898, p. 240); Nocard, Rous, and Dujardin-Beaumetz (Bull. de la Soc. Centr. de Med. Vet., 1899, p. 480).
- ³⁶Negri (Ztschr. f. Hyg., XLIII, 1903, p. 507).
- ³⁷Flexner and Noguchi (Jour. Exper. Med., XII, 1910, p. 227; XVIII, 1913, p. 461).
- ³⁸Flexnow and Associates (Jour. Infect. Dis., XX, 1918, pp. 281, 313, 345, 379).
- ³⁹Mallory and Medlar (Jour. Med. Research, XXXIV, 1916, p. 127).
- ⁴⁰Plotz (Jour. Am. Med. Assn., LXII, 1914, p. 1556); Plotz, Olitsky, and Baehr, (Jour. Infect. Dis., XVII, 1915, p. 1).
- ⁴¹Ferry (Jour. Path. and Bacteriol., XIX, 1915, p. 488).
- ⁴²Bullock and Craw (Jour. Hyg., IX, 1909, p. 35).
- ⁴³Betegh (Berl. Tierärzth. Wehnschr., XXVIII, 1912, p. 968).
- ⁴⁴Wolbach, Chapman, and Stevens (Jour. Med. Research, XXXIII, 1915, p. 117).
- ⁴⁵Novy and McNeal (Studies from Rockefeller Inst., VI, 1907, p. 375).
- ⁴⁶Wolbach and Binger (Jour. Med. Research, XXX, 1914, pp. 9, 23).
- ⁴⁷Borrel (Ann. de l'Inst. Pasteur, XVII, 1903, p. 123).
- ⁴⁸Marchoux (Compt. rend. Acad. d. sc., CXLVII, 1908, p. 357).
- ⁴⁹Moon (Jour. Infect. Dis., XIII, 1913, p. 165).
- ⁵⁰Poor and Steinhart (Jour. Infect. Dis., XIII, 1912, p. 203).
- ⁵¹Noguchi (Jour. Exper. Med., XVIII, 1913, p. 314).
- ⁵²Guarnieri (Arch. p. le sc. med., XVI, 1892, p. 403).
- ⁵³Brinckerhoff, Luzzier, and Councilman (Jour. Med. Research, XIV, 1906, p. 213).
- ⁵⁴Belin (Rev. Internat. de la Vaccine, IV, 1913, p. 128).
- ⁵⁵Steinhart, Israeli, and Lambert (Jour. Infect. Dis., XIII, 1913, p. 297).
- ⁵⁶Force (Jour. Lab. and Clin. Med., I, 1916, p. 243).
- ⁵⁷Wollstein (Jour. Exper. Med., XXIII, 1916, p. 353).
- ⁵⁸Feiling (Med. Chron., LXI, 1915, p. 142).
- ⁵⁹Dochez (Jour. Am. Med. Assn., LIX, 1912, p. 273).
- ⁶⁰Flexner and Noguchi (Jour. Exper. Med., XII, 1910, p. 227).
- ⁶¹Carter (Ann. Trop. Med. and Parasit., X, 1916, p. 163).
- ⁶²Hecker (Ztschr. f. Kinderh., II, 1911, p. 73).
- ⁶³Herman (Arch. of Pediat., XXXII, 1915, p. 503).
- ⁶⁴Mallory (Jour. Med. Research, X, 1904, p. 483).
- ⁶⁵Ross (Coll. Papers, John Howard McFaddin Researches, III, 1913, London Murray).
- ⁶⁶Class (Med. Rec., LVI, 1899, pp. 530-513).
- ⁶⁷Schultze (Med. Rec., LXXVII, 1910, p. 1046).
- ⁶⁸Ferry (Med. Rec., XCV, 1914, p. 934).
- ⁶⁹Pryer and Kelly (Jour. Lab. and Clin. Med., III, 1918, p. 269).
- ⁷⁰Ricketts and Wilder (Jour. Infect. Dis., IX, 1911, p. 9).
- ⁷¹Anderson and Goldberger (Pub. H. Bull., 86, 1912).
- ⁷²Gavino and Girard (Pub. del Inst. Bacteriol. Nazionale de Mex., 1910 and 1911).
- ⁷³Wolbach (Boston Med. and Surg. Jour., CLXVII, 1912, p. 419).
- ⁷⁴Meyer (Rep. 10th Internat. Vet. Cong., London, 1914).

Studies from the Research Laboratory.

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A STUDY OF THE CHARACTER OF THE FECES DUE TO VARIOUS FOODS IN CONNECTION WITH ANTHELMINTIC INVESTIGATION.

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In carrying on anthelmintic investigations, it is frequently important to know whether an anthelmintic is purgative or constipating in character, whether purgations given with anthelmintics are adequate or inadequate, etc. Certain diets are prone to cause free evacuation, while others tend to cause constipation. Since these facts should be considered in the administration of anthelmintics, I have kept some notes on dietary influences which are given for what general interest they may have.

Data for over 100 dogs in a series of 325 dogs used in anthelmintic investigations are available, as to the effect of the nature of the food on the character of the feces. The consistency and color of the feces were noted after 230 feedings which were given as follows: Bread, 86 feedings; cooked meats, which consisted of small particles of ground meat from which the bouillon had been extracted, 46 feedings; uncooked meat, usually beef heart and tongue, 46 feedings; bone with attached raw meat, 17 feedings; a combination of bread and uncooked meat, 31 feedings; a combination of bread and bone with attached raw meat, 4 feedings. The character of the feces on those days when factors other than the nature of the food seemed to be operative, is disregarded. Such factors, which might influence the fecal consistency and color, would be: (1) the anthelmintic used, since the anthelmintic itself usually imparts a decided color to the feces (a greenish color in the case of oil of chenopodium or a black color in the case of male-fern); (2) the purgative usually accompanying the anthelmintic, causing a diarrheal condition; (3) constipation, either due to the anthelmintic or to the animal's

unwillingness to defecate in its cage, or to other contributory factors: (4) intestinal disorders, often resulting in diarrhea, due to bacterial or other parasitic invasions; (5) various other pathologic conditions, such as obstruction to the flow of bile, diseases of the pancreas, etc.; (6) disturbance of the factors usually governing the fecal consistency, viz., the activity of secretion, of absorption, and the amount of water drunk.

Taking up these various feedings seriatim, the following are the outstanding features in regard to the consistency and color of the feces the day following feeding:

Bread: The characteristic feces following a bread feeding on the previous day are light yellowish to yellowish brown in color and loose to soft in consistency. After 86 bread feedings, the next day's feces were black and soft in 3 cases; greenish brown and soft in 22 cases; light yellowish to yellowish brown and loose to soft in 48 cases; greenish brown and solid in 6 cases; yellowish and fluid in 6 cases; black to brown and fluid in 1 case.

Cooked meat: The characteristic feces following the feeding of this finely ground, extracted meat are dark brown to black and fairly soft. Of the 46 cooked meat feedings, the following day's feces were: dark brown to black and fairly soft in 25 cases; greenish brown and hard in 3 cases; black to brown and fluid in 7 cases; brown to green and fluid in 11 cases.

Uncooked meat: Uncooked meat, which consisted chiefly of beef tongue and beef heart, usually caused a greenish brown, soft to fairly hard feces, often in compact lumps, the day following feeding. In the case of 46 feedings, 3 caused black and soft feces; 38 greenish brown, fairly hard feces; 4 brownish green, very hard feces, and 1 brownish green, fluid feces.

Bone with meat: Seventeen feedings of bone and attached raw meat were given, and in every case very characteristic clay-colored, brittle feces, appearing in small, compact lumps, were obtained.

Bread with raw meat: This mixed feeding of bread and raw meat caused characteristic greenish brown, rather soft feces. Of 31 feedings, 25 caused greenish brown, rather soft feces; 1 yellowish brown, soft and fluid feces; three greenish brown, hard feces; 1 green, fluid feces; and 1 brown, fluid feces.

Bone with meat and bread: Four such feedings were given,

and in each case characteristic clay-colored, brittle feces, in small, compact lumps, resulted.

DIET MAY BE DETERMINED BY FECES.

The above data shows that under normal conditions one can determine with a fair degree of certainty the diet on which an animal is kept, by means of the color and consistency of the feces. The usual yellowish, loose to soft feces due to the feeding of bread is usually greater in quantity than that of the harder, darker feces following a meat diet.

The softer consistency, i. e., greater water content, of the feces following a bread feeding is perhaps due to the stimulation of the mucous membrane by the undigested bread constituents, causing increased peristalsis and so hastening the transit through the intestine and thereby shortening the period of absorption. The amount of indigestible material in meat being materially less it would be logical to conclude that there would be more complete absorption and smaller residue, and hence a more compact, solid feces. The smaller amount of indigestible content in a purely meat diet would greatly prolong the intervals of defecation; whereas by the addition of bread to form a mixed diet, the intervals would be greatly reduced. We can also expect that inasmuch as bread has a greater amount of indigestible solids than meat, the quantity of feces following bread diet will be greater than that following a meat diet—that is, provided the same amounts of both foods are fed.

The color of the feces is also greatly affected by the nature of the food. On a meat diet, the color is dark, due to hematin and to ferrous sulphide, formed by the action on the hemoglobin derivatives of sulphuretted hydrogen generated by bacteria in the intestine. The usual clay-colored feces following the eating of bone are due to the calcium constituent present.

STUDY OF FECAL CONSISTENCIES IMPORTANT.

The study of fecal consistencies due to various foods is of importance to the veterinarian as well as to the medical man. It is a matter of importance to know whether the feces are such as would be expected after a particular diet, or are altered as a result of some particular condition. Its interest in the con-

duct of anthelmintic investigations lies in the fact that information can be obtained as to the effect on the feces of the anthelmintic in question in bringing about a change from the normal.

For example, the feces obtained after a therapeutic dose (0.1 mil per kilo) of oil of chenopodium, given with castor oil, is usually fluid in consistency and green in color in spite of the nature of the food fed. In the case of five dogs given a therapeutic dose of oil of chenopodium, three feedings of bread were given, after which feces of a yellowish, soft character would be expected; one feeding of meat was given, after which greenish brown, rather hard feces would be expected; and one feeding of bread and meat combined was given, after which greenish brown, rather soft feces would be expected; but in every instance the feces the day following treatment differed markedly from the usual and normal feces, being fluid in consistency and green in color.

The same was found to be true in the case of various chenopodium components¹ tested in our anthelmintic series. Dogs were given the following preparations, followed immediately by 30 mils of castor oil, fluid, green feces resulting in all cases on the following day.

Preparation No. 13 (the fraction of oil of chenopodium which distills up to 125° C. at 30 mm. of mercury) was given to eight dogs in the following dosage: one dog at 0.05 mil per kilo, two dogs at 0.074 mil per kilo, and five dogs at 0.1 mil per kilo. In four of the above cases, the dogs were fed on uncooked meat and in four cases on bread, the previous day.

Preparation No. 12 (the residue not distilling over at 145° C. at 30 mm. of mercury) was given to two dogs at the rate of 0.1 mil per kilo, one of the dogs being fed bread the previous day, the other being fed uncooked meat. Preparation No. 14 (the fraction not distilling over at 125° C. at 30 mm. of mercury) was given to four dogs as follows: one dog at 0.05 mil per kilo, one at 0.074 mil per kilo, and two at 0.1 mil per kilo. In each case the dog was fed uncooked meat the previous day. Preparation No. 16 (the residue not distilling over at 130° C. at 25 mm.

¹Hall, M. C., and Hamilton, H. C. Investigations of oil of chenopodium and the anthelmintic value of some of its components, *Jour. Pharm. & Exp. Therap.*, 1918, V. 11 (2), Apr., p. 281.

of mercury) was given to one dog at the rate of 0.1 mil per kilo. The animal was fed meat the preceding day. Preparation No. 19 (the residue not distilling over at 100° C. at 2 to 5 mm. of mercury) was given to one dog at the rate of 0.1 mil per kilo. The dog was fed meat the previous day.

Chenopodium itself is constipating in character, and in our experiments a purgative (castor oil) was administered to insure prompt passage of the anthelmintic through the digestive tract to lessen the local and systemic effects that may result from rapid absorption of the vermifuge. If chenopodium is administered in lethal doses, or in doses several times the therapeutic dose, with a purgative, such as castor oil, the constipating effect of the chenopodium very often masks the purgative effect of the castor oil, and hard, dark feces or no feces at all result the day following treatment.

This fact is exemplified by two dogs in our tests of chenopodium components. One dog was given Preparation No. 14, and the other Preparation No. 18 (the portion distilling below 100° C. at 2 to 5 mm. of mercury). In the first case the dog was given a lethal dose (0.6 mil per kilo) which was preceded by 30 mils of castor oil. The following day's feces were solid, hard, and dark brown instead of the expected fluid, green feces. In the second case the dog was given ten times the therapeutic dose (1 mil per kilo) which was administered with 60 mils of castor oil, 15 mils being given before, 15 with, and 30 mils after the administration of the drug. The following day no feces were obtained instead of the fluid, green feces one might expect if the purgative were operating effectively.

SUMMARY.

Soft, light-colored, plentiful feces are indicative of a bread diet. Dark, fairly hard feces in comparatively small amounts are indicative of a raw-meat diet. Very dark, fairly soft feces in small amounts are indicative of a finely chopped cooked-meat diet. (This seems to be especially true when the meat is fed while still warm.) Clay-colored, brittle feces in small lumps are indicative of some bone constituent in the diet.

Therapeutic doses of oil of chenopodium or distillation

products of oil of chenopodium, when given with castor oil, usually cause greenish, fluid feces, regardless of diet.

Excessive or lethal doses of chenopodium constituents cause constipation, in spite of therapeutic doses of castor oil, defecation being suppressed for a period of one or more days or the feces being hard and dark.

Studies from the Research Laboratory.

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A PHYSALOPTERA FROM THE DOG, WITH A NOTE ON THE NEMATODE PARASITES OF THE DOG IN NORTH AMERICA.

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In a series of over 300 dogs examined post mortem at Detroit, we found a single female specimen of *Physaloptera* in one dog, No. 300, an incidence of about 0.3 per cent. The head of the worm was so deeply and firmly imbedded in the duodenum about an inch below the pylorus, that we were unable to remove the parasite by what we regarded as a reasonable amount of traction without danger of breaking it. The worm and the tissue to which it was attached were, therefore, placed in normal saline solution for a time, and in the course of an hour the worm relinquished its hold and separated from the intestinal wall.

The description of a new species of nematode on a single female specimen is not a wholly satisfactory proceeding from the standpoint of those who describe such species or those who must consider their descriptions later. On the other hand, if it is desirable to describe the rare parasites of dogs, which parasites have considerable bearing on the subject of the dog as a carrier of parasites usually occurring in other hosts, then it is desirable that we have some name by which these parasites may be discussed. It simplifies discussion, and it is no serious matter that a parasite may be found to bear more than one name and that all but one of its names must be rated as synonyms.

On these grounds we are describing the nematode found by us in the dog as a new species. It is entirely improbable that this is a customary or even common parasite of the dog. It is probable, though not certain, that it is a parasite of some wild mammal, but it might even be a parasite of a bird or reptile, temporarily present in the dog after the dog had eaten the infested animal. In any case it is quite apt to be a species heretofore

undescribed, as there has been but little study of the genus *Physaloptera* on this continent.

Species *Physaloptera rara* Hall and Wigdor, 1918.

SPECIFIC DIAGNOSIS.—*Physaloptera*: Anterior extremity of the body somewhat attenuated. Cuticle strongly annulated (in the female at intervals of 50 to 200 μ), the first annulation behind the head forming a sort of collar, with the head somewhat sunk in the depression formed by this collar. Mouth with two large lateral lips (Figs. 1 and 2), each of which is prolonged anteriorly by 3 prominent teeth in a row, a somewhat smaller tooth being external to the middle tooth of the three. Each lip bears a pair of conspicuous papillae, one of the pair being situated near each end of the lip and toward its base, and a third papilla near the middle of the lip base. The esophagus length is 4.8 per cent of the total body length.

MALE unknown.



Figure 1 *Physaloptera rara*. Head. Lateral view.

FEMALE 24 mm. long, with a maximum diameter of 1.34 mm. The head is 90 μ long and 200 μ wide at the base. The collar in which the head is set is 324 μ wide. The teeth on the lips are about 12 μ long and about 10 μ wide at the base. The collar-like depression about the head is formed by the cuticle delimited by the first transverse striation. The third pseudo-annulus thus formed breaks on one side and runs obliquely back; this may be accidental (Fig. 3). The esophagus is 1.16 mm. long, the muscular portion being 526 μ long; the maximum width of the esophagus is 102 μ . The nerve ring surrounds the muscular esophagus at a point near its union with the glandular portion. The inconspicuous vulva is in the anterior portion of the body, 3.63 mm. from the anterior end. The vestibule of the ovejector (Fig. 4) proceeds inward a short distance and then turns toward the anterior end of the worm for a distance of about 0.6 mm.,

when it turns back past the vulva, the remainder of the genitalia lying in the posterior body between the vulva and the anus. The vestibule is $880\ \mu$ long. The succeeding portion, the unpaired portion of what Seurat calls the "trompe," which is the distal portion of the ovejector, is $2.16\ \text{mm.}$ long and is directed posteriorly throughout. This bifurcates and the 2 branches form a loop and meet the corresponding portions of the uterus. The most posterior ovarian loops (Fig. 5) are in the vicinity of the anal region. The anal aperture appears to be set deep in a depression formed by a fold of cuticle (Fig. 6). The external aperture of this cuticular depression is $420\ \mu$ from the posterior extremity of the body. The posterior extremity is comparatively blunt. This was the only specimen present, so there were no fertilized eggs in the worm.

HOST.—*Canis familiaris*.

LOCATION.—Duodenum.

LOCALITY.—Detroit, Michigan.

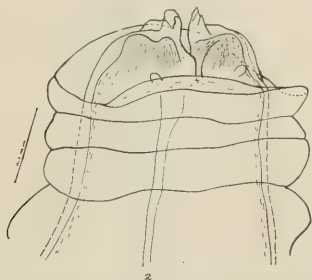


Figure 2. *Physaloptera rara*. Head. Dorso-ventral view.

Seurat (1917) divides the species of *Physaloptera* from mammals into 2 groups; in one group, such worms as *Ph. clausa*, the external tooth of the lip is approximately as large as the internal teeth, the latter forming a 3-pronged fork; in the other group, such worms as *Ph. abbreviata*, the external tooth is very large and the internal teeth are small and set at intervals. *Ph. rara* evidently belongs in the first group. The surprisingly short glandular esophagus distinguishes this species from the other species with which we have compared it wherever this feature is described for other species. It is unfortunate that we have for

study only a single specimen of the worm, as this feature is rather widely different from the corresponding condition in other species.

We take this occasion to summarize what is known in a general way of the nematode parasites of dogs in North America, thereby completing a series of papers on the parasites of dogs in North America. The other papers, published in recent issues of this journal, dealt with protozoan, cestode, trematode, acanthocephalid and arthropod parasites. While these records are not exhaustive, they are the first comprehensive summary of our knowledge of the parasites of dogs on this continent.

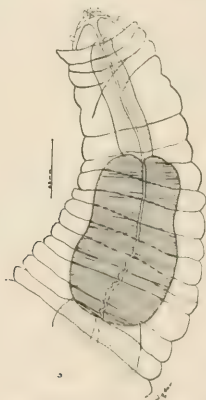


Figure 3. *Phascoleptus rana*. Anterior extremity.

NEMATODA. *Filaria osleri* was described from dogs in Canada by Osler (1877). It has since been found in Europe and has recently been reported again from this continent, after an interval of almost 40 years, by Milks (1916) at Ithaca, N. Y. The worm occurs in nodules in the trachea, bronchi and lungs.

Dirofilaria immitis was described from the heart blood of the dog in this country by Leidy (1856). The worm was subsequently found to be widely distributed over the world. It has been reported a number of times from the United States, being collected by Curtice, Hassall, Wheeler and others. It does not appear to be uncommon in the South, but it is evidently uncom-

mon in the northern United States. We have not found it in the post-mortem examination of over 300 dogs at Detroit.

Spirocerca sanguinolenta (*Spiroptera sanguinolenta*) has been reported from a tumor of the esophagus in a dog at Washington by Sommer (1896). More recently, Haythorn and Ryan (1917) have reported 6 cases of the occurrence of this parasite in dogs at Mobile, Ala., and note that the U. S. Bureau of Animal Industry has specimens from one case at Atlanta, Ga., and from 2 cases at Washington, one host said to be a lynx from the Zoological Park. (The other specimens at Washington are probably Sommer's.)

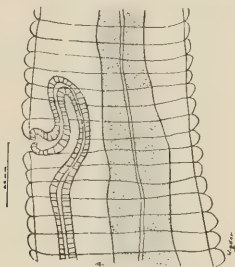


Figure 4. *Physaloptera rara*. Vulva region, showing loop of ovejector.

Trichuris depressiuscula, the whipworm, is a common parasite of dogs in the United States. We found it in 39.7 per cent of the first 300 dogs examined here by us, with an average of 21.4 worms per dog. The largest number present in a dog in this series was 677 and the next largest 299.

Trichinella spiralis has been experimentally developed by us in the dog here at Detroit.

Diocotophyme renale occurs in the kidney and abdominal cavity of the dog in the United States. The records of its occurrence in this country have been summarized by Riley (1916) and Hall (1916; 1917). Since the publication of these summaries, this parasite has been reported from the dog at Tama, Iowa, by Maxfield (1917), and in a paper by MacNider (1917) on nephropathy of the dog, we find the following: "At autopsy the kidneys of 4 of the animals were found to be the seat of infections by a parasitic worm, with surrounding areas of lymphocytic infiltration and connective tissue hyperplasia." These last cases are

from North Carolina, and we regard them as probably cases of *D. renale*.

The occurrence of *D. renale* was reported by Hall (1915) in 2 dogs out of 67 dogs examined post mortem at Detroit. In a continuation of that series of examinations, this parasite was subsequently found by us in Dog No. 242, a mongrel male with some characteristics of the rough-coated terriers, so that our percentage for the series of 300 is only 1 per cent. The worm found was a male and was located in a tough cyst in the pelvic cavity to the right of the urinary bladder. This appears to be an unusual location, as the worm is usually free, rather than encysted, when it occurs in the abdominal cavity.

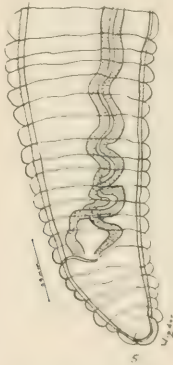


Figure 5. *Dioctophyme renale*. Posterior extremity, showing ovarian loops.

Maxfield's case, MacNider's 4 cases and our case make a total of 6 cases that should be added to the totals for the United States. In addition, we note the following from a letter to one of us (Hall) from Dr. Ralph W. Nauss of the California State Board of Health: "I note what you say regarding the prevalence of *Dioctophyme renale* among dogs in Chicago. This parasite was frequently found in dogs during my student days—1902-5—at the Northwestern University Medical College. On a later occasion, in 1911, when doing experimental surgery on dogs at this same institution, one case I remember distinctly of finding a female in the right kidney and a male (quite small in comparison with the one contained in the kidney) in the abdominal cavity

beneath the liver." The new cases listed here make it reasonably certain that this parasite has been found in the United States in at least 50 cases and probably in more than 50 cases. In a general way the distribution of the cases follows the Atlantic seaboard and the Great Lakes region, a distribution which is in accord with the supposition that the parasite has an intermediate stage in fish.

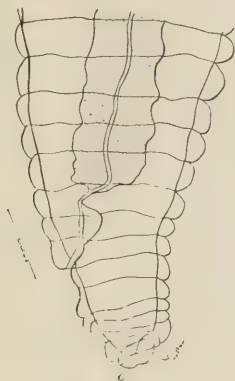


Figure 6. *Physaloptera rara*. Posterior extremity.

Ancylostoma caninum, the common dog hookworm, is generally distributed over the United States, though the available evidence indicates that it is more common in the South, as might be expected. However, it might be noted in passing that very little has ever been published regarding the parasites of southern dogs. We found hookworms in 33.3 per cent of our series of 300 dogs, with an average of 15.2 worms per dog. The largest number present was 282, and the next largest number was 165.

Uncinaria stenocephala has only been reported once, by Muldoon (1916), from the dog in the United States. Muldoon reported it from Ithaca, N. Y.; from correspondence it appears that no specimens of the worm were preserved. This is unfortunate, as the record was published without corroborative data and there is a possibility of misidentification.

Belascaris marginata is the roundworm which appears to be most common in dogs in the United States. We found ascarids

(the majority of them *B. marginata*) in 53.3 per cent of our 300 dogs, over half of them, with an average of 25.2 worms per dog. The largest number present was 2054, and the next largest was 204. Without this extremely large number, 2054, the average per dog was 12.3.

Toxascaris limbata appears to be much less common in this country than the foregoing species. Ransom (1913) reports *Toxascaris* from the dog, apparently at Chicago. We recently found specimens of this species fairly common in our ascarid material from dogs.

Agamemonatodum gaylordi was described from tubercles in the hyperplastic thyroids of experiment dogs at Craig Brook, Maine, by Ransom (1914).

HEAVY INFESTATIONS. Dog No. 170 had 2054 ascarids (*B. marginata*), most of them very small immature worms. Following anthelmintic treatment, 5 of these worms had been collected from the feces and 64 had been collected post mortem from the large intestine and cecum and credited to the efficacy of the anthelmintic. Of the remainder, 89 were found in the stomach and 1896 in the small intestine. Some of the very small larvae might have been passed in the feces and escaped detection, so that there is a possibility that there were even more ascarids present than the large number accounted for.

This dog was much emaciated, and had prominently enlarged thyroids. The animal received 2 anthelmintic treatments, the second 5 days after the first, and died the evening after the second treatment. Death was probably due to the cachectic condition of the animal, which in turn was apparently due to the gross infestation with ascarids, though the anthelmintic treatment in such a weakened animal may have hastened the end. Post mortem examination showed the following: The margin of the left lobes of the lung were congested; the external vessels of the heart seemed congested; the liver was cirrhotic; the spleen was dry and tough; the peripheral portion of the medulla of the kidneys was hyperemic; the bladder was distended; the gastric mucosa was gelatinous and showed some dark areas that apparently had been hemorrhagic; the jejunum showed locally inflamed areas and the ileum was inflamed and had some local hemorrhages; the colon was inflamed, the rectum hemorrhagic, and the glands of the rectum and cecum were very prominent.

Some of the young ascarids collected from this dog were

put in Kronecker's solution (a slightly alkaline physiologic saline solution), where they lived for 64 hours with a room temperature of about 26° C. The adults of this dog ascarid were found by Hall (1917) to survive for 14 days in Kronecker's solution.

To get some information in regard to the developmental period of these ascarids, 30 young worms, none of them more than 1 cm. long, were put in gelatine capsules and fed to Dog No. 173. The feces of this dog had been examined for the five-day period previous to the feeding and once 3 days before and no worm eggs of any sort had been found. Ascarid eggs first appeared in the feces 11 days after the feeding the worms to the dog, indicating that the female ascarid of the dog attains maturity and begins egg production within 11 days after reaching a length of about 1 cm. This dog was killed 48 days after these ascarids were fed to it. During that time the dog had vomited 3 ascarids on one occasion and 7 on another occasion. On post-mortem examination the dog had 83 ascarids, which with the 10 worms vomited makes a total of 93 ascarids following the feeding of only 30 ascarids. We may assume that immature worms were present during the period when fecal examination showed the absence of eggs, or that the dog acquired the additional worms from eggs in the feces as a result of the experimental infestation, or that the dog acquired the additional worms from eggs present in the cage from previously infested dogs. Experiments noted below indicate that the second assumption is a possibility.

The experiment in infecting this dog with ascarids is of interest in connection with the work of Stewart (1916-1917) and of Ransom and Foster (1917), on the life history of ascarids. Stewart demonstrated experimentally that when infective eggs from ascarids of man or of swine were fed to rats or mice, the embryos would escape from the eggs in the digestive tract and subsequently appear in the liver, spleen and lungs, later stages apparently migrating from the lungs up the trachea to the mouth and ultimately being swallowed in saliva and attaining the intestines, where they did not develop, but passed out in the feces. In view of the general failure which had followed most of the attempts to infect animals directly with infective eggs of suitable ascarids, Stewart concluded that rats and mice acted as intermediate hosts of ascarids, the infective larvae escaping in the saliva or feces of the rat and presumably attaining their definitive host in contaminated food or water. Ransom and Foster have stated

that Stewart's conclusions do not necessarily follow from his findings, and we suspect that most helminthologists at present would agree with Ransom and Foster in this, even though inclined to give all due credit to Stewart for what is evidently a very interesting and valuable piece of work. Ransom and Foster have confirmed Stewart's work experimentally and have obtained similar results with guinea pigs. They further note one case where a young pig was fed ascarid eggs and died a week later; numerous ascarid larvae were found in the lungs, trachea and pharynx. They correlate this finding with Epstein's (1892) success in developing *Ascaris lumbricoides* of man by feeding eggs to young subjects, and state that age is an important factor in determining susceptibility to ascarid infestation.

In the case of Dog No. 173, the animal was in a wire cage with a solid metal bottom and the cage was placed on top of some similar, but larger, cages which usually contained dogs. We consider this a location that would not be much disturbed by rats or mice which might infect the food or water of the dog in the cage, especially as the building is a concrete rat-proof structure with practically no place for rats or mice to hide except in the dog cages. The only food that might have come in contact with rodents is the stale bread, but this is furnished in entire loaves, free from rodent feces and, so far as we have noticed, apparently free from tooth marks that might indicate the presence of rodent saliva. The indications are that this dog acquired his infestation with worms in excess of the 30 originally fed to it, by contamination of food or water with eggs from the original worms.

In this connection it might be noted that Ransom and Foster call attention to the fact that heavy infestations of the lungs in rats and mice produce a serious pneumonia which is frequently fatal, and conclude that it is not improbable that ascarids are frequently responsible for lung troubles in children, pigs and other young animals. In the case of Dog, No. 170, there was a congestion of the margins of one lung found post mortem, as noted, but it is difficult to correlate this with the ascariasis. The recent invasion of the lungs by about 2000 ascarids should theoretically give rise to a generalized pneumonic condition, if anything, rather than a congestion confined to the margin of the left lobes; still, inflammation might have occurred and subsided. We do not disagree with their conclusions and we regard the ascarid as a dangerous parasite, but in this case the presence of

so many young ascarids in the intestine with a lack of the lung condition which might be expected suggests to us that the injury to the lung in the case of the rat and mouse fed with human or pig ascarids is partly due to the fact that these worms were in an unusual host, a condition which often leads to added injury. Thus in our experience trichinae in rats do not give the thermal and other clinical conditions found in man, the reaction in man, the unusual host, being much more severe. It is also true that the large clinical experience of the world in dealing with so common a parasite as the ascarid has credited it with the production of a wide range of symptoms in connection with the gastro-intestinal tract and the nervous system, but pneumonic conditions, except from the invasion of the lung by the wandering adult worm, do not seem to have been associated with it. Of course, clinical experience may be at fault here, as it has been in other instances.

In connection with the well-known wandering habits of the adult ascarid, it may be noted that this habit of entering the ducts of the pancreas and the liver and of traveling up the esophagus and leaving the pharynx by way of the nares, the trachea or the Eustachian tubes is apparently much more common in the case of the ascarids of man and swine than in the case of the ascarids of the dog. We have only seen one such case in the dog. This animal, a six-months-old pup, was found dead one morning with an ascarid projecting from one of the anterior nares. Behind it was another worm. On removing the head, another ascarid was found in the pharynx and posterior nares. Another worm was found in one bronchus. There were 19 worms in the intestine. Apparently death was due to these ascarids. The possibility of post mortem wandering cannot, of course, be absolutely excluded, but in our experience post mortem wandering of parasites in the dog must be a very rare thing, as we have almost no evidence of it.

We had one case of severe infestation with whipworms, a rough count showing approximately 677 worms, of which 421 were in the cecum and 253 in the colon and rectum to within 3 inches of the anus. In the cecum there was a mild hyperemia associated with the attachment of the worms.

MULTIPLE INFESTATIONS. Of our 300 dogs, 156 had only one kind of nematode worms, 91 had 2 kinds, 21 had 3 kinds, and none had more than 3 kinds.

CULTURE METHODS FOR EGGS. The culture method recommended for coccidia by Cole and Hadley (1910), the use of a 10-per-cent potassium bichromate solution, gave us very good results in culturing ascarid and hookworm eggs. At room temperatures of 20 to 29° C. ascarid eggs would form the two-celled stage overnight. In 4 days embryos could be seen moving about in the eggs. Ascarid eggs kept under the same conditions, but in a solution of tap water, showed the two-celled stage in about half the eggs in 18 days, the other half being still in the one-celled stage.

Hookworm eggs in the potassium bichromate solution at room temperatures of 20 to 23° C. showed actively motile embryos within 36 hours.

BIBLIOGRAPHY.

- FESSEIN, ALDIS. 1892. Ueber die Uebertragung des menschlichen Spulwurms (*Ascaris lumbricoides*). *Verhandl. Versamml. Gesellsch. Kinderh. Deut. Naturf. u. Aerzte*, v. 9, pp. 1-16.
- HALL, MAURICE C. 1916. American records of *Discothyma renale*. *J. Am. Vet. Med. Assoc.*, v. 3 (3), Dec., pp. 370-371.
- HAYTHORN, S. R., and A. H. RYAN. 1917. Aortic aneurisms in dogs with the report of six cases. *J. Med. Research*, v. 35 (3), Jan., pp. 411-423, pls. 28-29.
- LEIDY, JOSEPH. 1850. Descriptions of three filariae. *Proc. Acad. Nat. Sci., Phila.*, v. 5 (6), Nov. Dec., pp. 117-118.
- MACNIDER, WM. DEB. 1916. A pathological study of the naturally acquired chronic nephropathy of the dog. Part 1. *J. Med. Research*, v. 34 (2), May, pp. 177-197, pls. 7-9.
- MANFIELD, FRED M. 1917. Common parasites of the digestive tract. *Am. J. Vet. Med.*, v. 12 (5), May, pp. 295-297; discussion on pp. 298-300, 314.
- MILKS, H. J. 1916. A preliminary report on verminous bronchitis in dogs. *Rept. N. Y. St. Vet. Coll. for yr. 1914-1915*, pp. 129-135, 2 pls.
- MULLENDEN, W. E. 1916. Uncinariasis in dogs. *Rept. N. Y. St. Vet. Coll. for yr. 1914-1915*, pp. 136-141.
- OSLER, WILLIAM. 1877. Verminous bronchitis in dogs. *Veterinarian, Lond.*, (594), v. 50, 4 s., (270), v. 23, June, pp. 387-397, 2 figs.
- RANSOME, BEAVER H. 1913. *Cysticercosis*, the cause of tapeworm cysts in mutton. *J. Agric. Research*, v. 1 (1), Oct. 10, pp. 15-58, pls. 2-4, 13 text figs.
1914. (*Agamonematodum gaylordi*). In *Bull. Bu. Fisheries, Wash.*, Doc. 790, v. 32, Apr. 22, pp. 509-501, 1 fig. 125.
- RANSOME, BEAVER H., and WENDICE D. FOSTER. 1917. Life history of *Ascaris lumbricoides*. *J. Agric. Research*, v. 11 (8), Nov. 19, pp. 395-398.
- RILEY, WILLIAM A. 1916. The occurrence of the giant nematode, *Discothyma renale* (*Eustrongylus*) in the United States and Canada. *J. Am. Vet. Med. Assn.*, v. 2 (6), Sept., pp. 801-809.
- SIEKA, J. G. 1917. Physalopteres des mammiferes du Nord-Africain. *Compt. Rend. Soc. d. Biol., Paris*, v. 80 (4), pp. 210-218.
- SOMMER, H. O. 1896. Results of an examination of fifty dogs, at Washington, D. C., for animal parasites. *Vet. Mag.*, v. 3 (8), Aug., pp. 483-487.
- STEWART, F. H. 1916. On the life history of *Ascaris lumbricoides*. *Brit. Med. J.*, (1896), v. 2, pp. 367, 3 figs.
1916. The life history of *Ascaris lumbricoides*. *Brit. Med. J.*, (1896), v. 2, p. 474.
1916. Further experiments on *Ascaris* infection. *Brit. Med. J.*, (1916), v. 2, pp. 486-488.
1916. On the life history of *Ascaris lumbricoides*. *Brit. Med. J.*, (1916), v. 2, pp. 753-754.
1917. On the development of *Ascaris lumbricoides* Lin. and *Ascaris suilla* Duj. in the rat and mouse. *Parasitology*, v. 9 (2), pp. 213-227, 9 figs., 1 pl.

Studies from the Research Laboratory.

Parke, Davis & Co.

Reprint No. 173, 1918.

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SOME STUDIES OF *BELASCARIS MARGINATA* AND *TOXASCARIS LIMBATA*, INCLUDING A SIMPLE METHOD OF DIFFERENTIATING THEM.

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Belascaris marginata and *Toxascaris limbata* are the two common ascarids reported from the dog in this country. *Belascaris* is probably encountered more frequently in our experience, but *Toxascaris* is not as rare as has been believed. Hall (1917) on the strength of findings from 47 Michigan dogs infested with ascarids, states: "All the worms that I have examined have been *Belascaris marginata*, which is also the common form at Washington, D. C. The other species, *Toxascaris limbata*, is apparently the less common form in this country." There is, however, available to us material from approximately 128 more ascarid-infested dogs obtained from the Detroit city pound, and a close scrutiny of the material at hand discloses a greater proportion of *Toxascaris* than was expected.

Of the two species *Toxascaris* apparently is the smaller, the females ranging from 6.13 cm. in length and the males from 4.6 cm. *Belascaris* females are usually 6-18 cm. in length and longer (Dr. Hall tells me that he has collected specimens 21 cm. long), and the males usually attain a length of 5-10 cm.

Leiper (1907) proposed the genera *Belascaris* and *Toxascaris*. The generic characteristics given for them by Gedoelst (1911) are:

Belascaris: Lips with lateral lobes prolonged into a digitiform, incurved lobule; submedian cephalic papillae with a voluminous, ellipsoidal base bearing two papilliform points; posterior extremity of male terminated by a stout conical appendage; caudal wings more or less evident; two subequal spicules provided with two wings forming a channel; vulva towards the

anterior fourth of the body; ovaries and oviducts extend anterior of the vulva; main branch of the uterus long; eggs more or less globular, with a thin shell hollowed with pits.

Toxascaris: Lips with lobules separated from the lobes by a deep furrow, enlarged and bilobed at their termination; submedian papillae of medium thickness; posterior extremity of male without wings, terminating in a point; spicules slightly unequal in the form of channeled tubes, without wings; vulva toward the anterior third of the body; main branch of the uterus very short; eggs subglobular, with thick, smooth shell.

Leiper (1907) makes the following differentiation:

Belascaris, type *Ascaris mystax*: Anterior end of the body in preserved specimens bent ventrally. Cuticle coarsely striated. Esophagus with distinct bulbous portion posteriorly containing nuclei of esophageal glands. Tail of the male probular—that is, having an outline similar to that of a closed fist with the forefinger semi-extended. On the bulging portion immediately behind the anus is a pair of single papillae with sunken caps, and on the finger-like tail two further pairs ventrally and two laterally, the tips of which apparently, from preserved specimens, support a slight expansion of cuticle. The coils of the testis are situated in the anterior half of the body cavity, differing in the various species in the limit of their forward extension. There is a remarkably long "vesicula seminalis," and short ejaculatory duct. In the female the genital opening is situated in the anterior part of the body and the eggs have a corrugated shell.

To this genus belong *Ascaris mystax* (Zeder), *Ascaris triquetra* (Rudolph's type specimens), and also a large new species from the Egyptian fox. From the brief published description of *Ascaris globulus* (v. Linstow) this species may be ascribed to the genus, as may also *Ascaris leptoptera* (Rudolphi).

Toxascaris, type *Ascaris leonina* (v. Linstow):

The anterior end of the body is, in preserved specimens, bent dorsally. The cuticle is finely striated. The esophagus is simple and opens directly into the intestine without any intervening bulbous portion. The palps of the lips are distinctly club-shaped terminally. In the male the tail tapers to an acicular tip without any ventral retrusion behind the anus. The post-anal papillae are arranged into two groups, a ventral set of paired papillae

continuous in line with the pre-anal row on each side of the body, and a lateral set, arranged, as it were, one at each corner of a triangle on the outer aspect of the tail. In all there are six pairs of post-anal papillae, three lateral and three ventral, and of the ventral the pair succeeding the anus is double. The coils of the testis lie in the anterior part of the posterior half of the body. The tubular seminal vesicle is long, though short relative to that in *Belascaris*. The ejaculatory duct remains short. The genital opening of the female is found at about the center of the body and the eggs are oval and have a smooth capsule. The genus thus defined comprises the species: *Ascaris marginata* (Rudolphi), *Ascaris leonina* (v. Linstow), *Ascaris tigridis*, Gmelin), and a further species from the Sudan not yet published.

Although all the above characters can be ascertained with certainty in differentiating the two species under consideration, certain features are especially easy to observe and compare. In females of *Toxascaris* the vulva is in the approximate anterior third of the body, the ovaries and oviducts not extending anterior to that point. In females of *Belascaris*, on the other hand, the vulva is approximately in the anterior fourth of the body and the ovaries and oviducts extend well beyond the vulva, often almost up to the lips.

This conforms closely with Geddoelst's (1911) differentiation of the two species in regard to the position of the vulva, but differs from Leiper's (1907) differentiation which places the distance of the vulva in *Toxascaris* at the middle of the body. As Leiper's type *Toxascaris* species *Ascaris leonina* (v. Linstow) is not available for study, I cannot pass on the validity of his distinction in regard to that species, but the location as stated is not a generic character as it does not hold true for the included species, *T. limbata*. Tentatively I suggest that the vulva in *Toxascaris* is not located in the middle of the body but anterior of this to a point one-third of the body length from the head.

To determine the variations occurring in the extent of the female genitalia in both the *Toxascaris* and *Belascaris* forms, twenty-five female specimens of *B. marginata* and *T. limbata* were taken at random and the total body length and the distance from the anterior end of the genitalia to the anterior extremity of the body ascertained, with the following results:

	<i>Toxascaris</i> <i>limbata</i>	<i>Belascaris</i> <i>marginata</i>
Average length	92.5 mm.	112.5 mm.
Average distance of genitalia from anterior extremity.....	31.2 mm.	13.8 mm.
Average relation of distance of genitalia from anterior end to total length of body in per cent.....	34.1%	11.2%

From the above table of average data, it can be seen that the female genitalia in *Toxascaris* extend approximately into the anterior one-third of the body, while in *Belascaris* they extend approximately into the anterior one-ninth of the body. Thus a female specimen can be macroscopically determined as *Toxascaris* or *Belascaris* without resort to a detailed study, as the position of the genitalia can be readily made out through the translucent body wall.

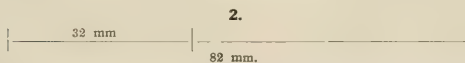
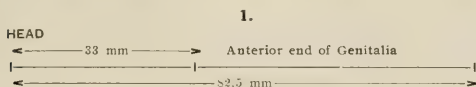
The diagrams below illustrate graphically some extreme and average female *Toxascaris* and *Belascaris* in the series of 25 of each species studied, showing their total length and distance from head to anterior end of genitalia.

Aside from these characteristics, *Toxascaris* and *Belascaris* differ quite markedly in respect to their general color and the esophageal and egg structure. *Toxascaris*, milky-white in appearance, contrasts with the usual pale butter-yellow *Belascaris*. This color distinction still persists after the worms have been kept in formaldehyde for a long period. The esophagus is distinctive for the two species. *Belascaris* has a bulb-like posterior expansion of the esophagus (Fig. 1), while *Toxascaris* has no such expansion but a direct simple communication with the intestine (Fig. 2). The eggs also offer suitable material for differentiation. *Toxascaris* eggs in utero usually measure 72-90 μ in length and 64-76 μ in width, while those of *Belascaris* in utero usually average 72-104 μ in length and 50-78 μ in width. *Toxascaris* eggs in the feces average 74-104 μ in length and 66-80 μ in width, and those of *Belascaris* in the feces average 72-94 μ in length and 60-72 μ in width. The most striking characteristic, however, is not the difference in the size of the eggs, but in their general appearance.

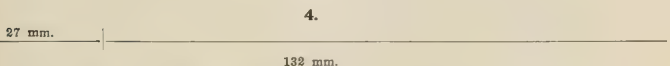
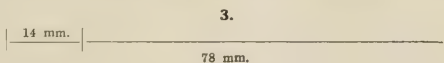
Toxascaris eggs are usually ellipsoidal in shape, clear and smooth in appearance and possess an outer clear double-contoured chitinous shell and an inner yellowish membrane which is marked with interlacing striations giving the suggestion that this membrane is composed of interlaced fibers. (Figs. 3-5.) This

inner membrane is apparently laid down in the upper region of the ovejector. *Belascaris* eggs, on the other hand, are usually sub-globular, and are very dense, being covered with an albuminous coat which is entirely lacking in the *Toxascaris* egg. This albuminous coat is external to the chitinous shell, as in *Ascaris lumbricoides*, and is mammillated at regular uniform intervals (Figs. 6-8). In *Ascaris lumbricoides*, the albuminous layer gives an irregular contour to the egg.

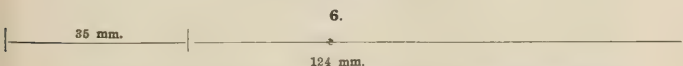
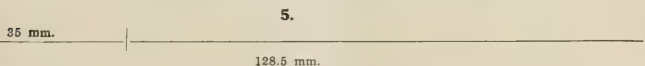
The eggs of both species are highly resistant, this being especially true in the case of the *Toxascaris* egg. Eggs were obtained from the uteri of both *Toxascaris* and *Belascaris* which had been kept for a long period in a formalin-alcohol preservative, and



Diagrams No. 1 and No. 2 illustrate the extreme posterior extent of the genitalia in *Toxascaris*. In No. 1 the genitalia extend into the anterior 40% of the body, in No. 2 into the anterior 39% of the body, or approximately into the anterior 2/5 of the body in both cases.



Diagrams No. 3 and No. 4 illustrate the extreme posterior extent of the genitalia in *Belascaris*. In No. 3 the genitalia extend into the anterior 18% and in No. 4 into the anterior 20% of the body, or approximately into the anterior 1/6 and 1/5 of the body respectively.



Diagrams No. 5 and No. 6 illustrate the extreme anterior extent of the genitalia in *Toxascaris*. In No. 5 the genitalia extend into the anterior 28% and in No. 6 into the anterior 29% of the body, or approximately into the anterior 3/10 of the body in both cases.

7.

6.5

102 mm.

8.

5.5

91 mm.

Diagrams No. 7 and No. 8 illustrate the extreme anterior extent of the genitalia in *Belascaris*. In both cases the genitalia extend into the anterior 6% of the body, or approximately into the anterior 1/16 of the body.

9.

36

108 mm.

10.

27

80 mm.

Diagrams No. 9 and No. 10 illustrate the average anterior extent of the genitalia in *Toxascaris*. In No. 9 the genitalia extend into the anterior 33.3% and in No. 10 into the anterior 33.7% of the body, or approximately into the anterior 1/3 of the body in both cases.

11.

26

142 mm.

12.

15

132 mm.

Diagrams No. 11 and No. 12 illustrate the average anterior extent of the genitalia in *Belascaris*. In both cases the genitalia extend into the anterior 11% of the body, or approximately into the anterior 1/9 of the body.

found to have undergone development, with the formation of well-developed embryos in *Toxascaris* eggs (Fig. 9) and with segmentation (Fig. 5) in *Belascaris* eggs. The inner membrane present in the *Toxascaris* eggs in the feces is apparently rendered homogeneous or dissolved when kept in formalin-alcohol, as it is not visible in such preserved material. This was noted in *Toxascaris* eggs which had undergone development in utero when kept in formalin-alcohol and also in *Toxascaris* eggs which had been reared and preserved in alcoholic solutions of various strengths. *Toxascaris* eggs showed embryo development in 3 days at room temperature (26°-33° C.) in alcoholic solutions, to which 5 per cent potassium dichromate was added, in varying strengths up to 70 per cent, while the eggs were killed and preserved in a 75 per cent solution. In every case the inner membranous coat became invisible.

The resistance to formaldehyde of ascarid eggs has been previously noted by several workers. Foster (1916) states: "It is a well-known fact that in the case of several species of parasites, the ova of which are characterized by a relatively thick egg shell, the eggs are affected but little, if at all, by formalin solutions. Ascarid eggs, for example, may be kept alive for months or even years, in formalin. Morris when examining some human feces which contained many eggs of *Ascarid lumbricoides* and which had been preserved in a 2 per cent solution of formalin for two years, found that some of the eggs contained actively motile embryos. Four months later there was an apparent increase in the number of eggs containing embryos. In my own experience it has been found that a formalin solution is a very satisfactory medium in which to incubate ascarid eggs, as it prevents the growth of molds, bacteria, etc. I have been able to develop *Toxascaris* eggs in a 40% formalin solution. Eggs cultured in a 35% formalin solution with about 5 per cent potassium dichromate added and kept at room temperature (26°-31° C.) showed embryo development in three days.

In this connection it might be noted that *Toxascaris* eggs collected from the feces were cultured in a 10 per cent potassium dichromate solution, which offers a very suitable medium for ascarid egg development, as it does for other helminth eggs and for coccidia. At the end of a period of 2-3 days at room temperature (26°-33° C.) embryos were noted. (Fig. 5).

A peculiar *Toxascaris* egg (Fig. 12), possessing an unusually broad, clear, transparent outer membrane, was obtained from the feces of one dog, No. 311.

The writer gratefully acknowledges the advice and assistance of Dr. Maurice C. Hall in the preparation of the foregoing paper.

REFERENCES.

- FOSTER, W. D., 1916. A further note on polyradiate cestodes. *Science*, n. s., 45, No. 1183, 388-389.
 GEDOELST, L., 1911. *Synopsis de Parasitologie de l'Homme et des Animaux Domestiques*. Liège et Bruxelles XX, 332 pp., 327 figs.
 HALL, M. C., 1916. Parasites of the dog in Michigan. *Journal A. V. M. A.*, 51, n. s., 4, No. 3, pp. 383-396.
 LEIPER, R. T., 1907. Two new genera of nematodes occasionally parasitic in man. *British Medical Journal*, 1, pp. 1296-1298.

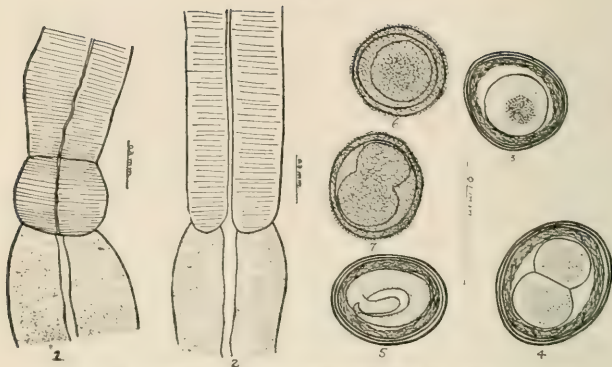


Fig. 1. *Belascaris marginata* showing esophagus and intestine.

Fig. 2. *Levinscaris limbata* showing esophagus and intestine.

Fig. 3. Eggs of *T. limbata* from feces.

Fig. 4. Eggs of *T. limbata* from feces cultured in 10 per cent potassium dichromate, showing division.

Fig. 5. Egg of *T. limbata* from feces cultured in 10 per cent potassium dichromate, showing embryo.

Fig. 6. Egg of *B. marginata* from feces.

Fig. 7. Egg of *B. marginata* from uterus, showing division.

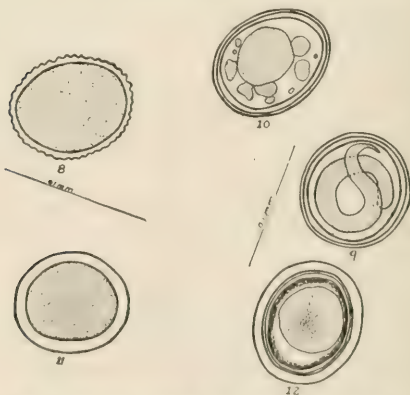


Fig. 8. Undeveloped ovum of *B. marginata* from uterus.

Fig. 11. Undeveloped ovum of *T. limbata* from uterus.

Fig. 9. Egg of *T. limbata* from uterus, showing embryo.

Fig. 10. Egg of *T. limbata* from uterus.

Fig. 12. Peculiar egg of *T. limbata* from feces.

**Studies from the Research Laboratory.
Parke, Davis & Co.
Reprint No. 174, 1918.**

(Reprinted from *The Creamery and Milk Plant Monthly*, Vol. VII, No. 10, Oct., 1918, pp. 59-61.)

STABLE DISINFECTION.*

BY H. PRESTON HOSKINS, V.M.D.,

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Strictly speaking, stable disinfection is only one small phase of the very much bigger and more complex subject—milk hygiene. However, we should regard stable disinfection as one of the cogs in the big wheel of clean milk production, and if there is anything wrong with this cog, the machine does not work smoothly, and it is only a question of time how long the machine can run before it must be shut down completely for repairs.

It is now almost half a century since Lister introduced the antiseptic treatment of wounds. Modern methods of sanitation can be traced back directly to Lister's discovery. Phenol, more commonly known as carbolic acid, was the first antiseptic used by Lister, and it is still in use today, but like the majority of such substances, it has its disadvantages with its advantages, its undesirable features with those that are desirable.

It is a common practice to measure the strength of a disinfectant as compared with carbolic acid as a standard. When we say that a certain disinfectant has a phenol coefficient of 5, we mean that under identical conditions the disinfectant in question will be five times as powerful in destroying germ life as carbolic acid, or its germicidal value is five times as great as carbolic acid.

A slight distinction is usually made between a disinfectant and an antiseptic. We say that an antiseptic will retard or even entirely stop the growth and multiplication of bacteria, but it will not be strong enough to actually kill the bacteria in question. On the other hand, a disinfectant must be strong enough to actually kill bacteria before it can qualify as a disinfectant.

*Address before the Michigan Milk and Dairy Inspectors' Association, Saginaw, Feb. 5, 1918.

From these definitions it will be seen that all disinfectants would be antiseptics, although the reverse would not be true. It will also be seen that a certain substance might be a disinfectant in a certain dilution, and in a higher dilution or a weaker solution it would be only an antiseptic. The terms germicidal and bactericidal are substantially synonymous with the term disinfectant.

There is quite a variety of disinfecting agents. These might be divided, for convenience, into three groups, viz: sunlight, heat in its various forms, and chemical substances.

Sunlight is always referred to as the cheapest disinfectant at our disposal. This fact should be kept constantly in mind in the construction of dairy barns or stables of any kind where animals are kept. The maximum window area allowable with good construction should be provided for, and where there is a choice, and there usually is, windows on the south side of the barn are preferable to any other one side. This fact was in the minds of the officials who drew up the dairy score card, in allowing a generous number of points to the dairyman whose barn was well provided with windows. A minimum of four square feet of window area for each cow stall should always be provided.

Besides allowing the entrance of sunlight which in itself is always desirable, the illumination afforded by the windows will undoubtedly result in the stable being kept in cleaner condition. Dirt that is rendered visible is much more likely to be promptly removed than the filth which slowly but surely accumulates in the dark corners.

Now, the dirt itself is not particularly harmful or dangerous, but for the fact that it may, and frequently does, afford either a resting place or a breeding place for disease-producing germs. There are three things that disease-producing bacteria usually require for growth: food, moisture and a suitable temperature, usually that of the body.

With other bacteria, not necessarily disease-producing, the body temperature is not actually required, but they multiply at ordinary atmospheric temperature within certain limits. These bacteria are the ones that bring about the decomposition of organic matter, the putrefactive and fermentative groups of

organisms. The putrefaction of animal and vegetable matter is usually accompanied by the liberation of gases, many of which have objectionable odors. Such a condition should never be tolerated in or near any place where milk is handled.

Heat in its various forms is another disinfectant at our disposal, and one that we should utilize wherever possible. We are depending upon heat as a disinfectant every time we cremate the carcass of an animal that has died from an infectious disease, every time that we scald out a milk pail with boiling water, or every time we sterilize a milk can by inverting it over a jet of live steam. Pasteurization is merely a process of utilizing moderate heat for a given length of time, in preference to a higher degree of heat. As a general principle, the higher the temperature the shorter the time required to destroy a given amount of infection.

For example, to destroy the tubercle bacillus in milk the usual time and temperature combinations given are:

140 degrees for 15-20 minutes

160 degrees for 5-10 minutes

176 degrees for 1-2 minutes

The chemical disinfectants might be divided into three groups, according to their material state, namely, solids, liquids and gases.

Certain gaseous disinfectants, of which formaldehyde is an example, are excellent disinfecting agents, but only when the proper conditions are provided. Disinfecting by means of a gas is usually spoken of as fumigating, but from the very nature of the process its success depends almost entirely upon our ability to properly confine the disinfecting gas. This is a difficult matter in the average stable, and the time and expense of tightly sealing up all the openings in the stable would probably offset any advantages the method might possess.

Solids almost without exception must be combined with more or less moisture before they are able to exert their disinfecting power. Therefore, in looking about for a suitable disinfectant for use in the stable, we are limited somewhat in our choice. Besides the physical nature of the material itself, we must take into consideration its cost and its adaptability for the particular purpose in mind.

Some very good disinfectants are highly poisonous, and must be used only with considerable caution, notably carbolic acid and bichloride of mercury (corrosive sublimate). The latter substance has one great advantage in that it is practically odorless. Against this we have its poisonous nature, and the fact that it corrodes metals. We might go down the entire list of chemicals that have disinfectant properties without finding a single one that did not have some great drawback to its extensive use as a general disinfectant.

The disinfectants usually spoken of as the coal-tar group, a large number of which are available, are generally regarded by most authorities as the best for general disinfecting purposes.

It should be kept in mind that the germicidal power of a certain substance may vary with the organisms it is called upon to destroy. This is due to the resistance to destruction offered by these bacteria. That class which forms spores—anthrax, blackleg and tetanus, for example—are extremely hard to kill with any disinfectant when they are in the spore stage.

Carbolic acid in strength sufficient to kill many bacteria will not destroy the germ that causes hog cholera. On the other hand, we have examples where certain germs appear to be highly susceptible to the action of certain chemicals. These facts should be kept in mind and we should take advantage of our knowledge whenever we are called upon to advise or to put into practice the theories of disinfection.

There are several diseases which are commonly met with in our dairy herds, caused by bacteria concerning which we have considerable knowledge, the fruits of time-consuming and pains-taking study and investigation. This knowledge should be of the greatest possible use to us in the intelligent performance of our duties.

In the matter of infectious diseases of our food-producing animals, and the ultimate eradication of these plagues from the face of the earth, if such a thing is possible, we must learn to regard the diseased animal as the greatest menace to the healthy animals in the herd, whether the disease be tuberculosis, infectious abortion, hog cholera, glanders or any other disease of like nature.

A great deal of the time, labor and expense of disinfecting

may be for naught if the source of additional infection is allowed to remain. For instance, if a herd of cows is tested for tuberculosis, and a number of them react, unless all of these reactors are removed from the herd immediately and either destroyed or isolated in a quarantine barn, there is little to be gained by the test or any disinfection of the stable subsequent to the test, as far as the ultimate eradication of the disease is concerned.

You will note that I was careful not to say that there would be no use in such a procedure. I have always been of the opinion that no matter how hopeless a job of disinfecting might appear, if real effort were intelligently expended in the work a great deal of the existing infection would probably be destroyed, and the chances for further damage made just that much less. In this connection it might be said that many disease germs probably do not multiply to any great extent outside of the animal body. However, there is no telling how long they may lurk around, awaiting the time when they will again find their way into the body of a susceptible animal, there again to renew their activities along their particular line.

Much has been said and written on the construction of dairy barns, and it is pleasing to note the tendencies of the present day toward so building these structures that they can be easily cleaned and kept clean. The floors should receive special attention in this regard. There is much to be said in favor of concrete construction, in spite of the fact that it is frequently objected to on the score that concrete floors are cold and damp in winter and give rise to diseases of the udder. Creosote blocks set in concrete do not have this objectionable feature, are almost as durable and not much more expensive.

Stable disinfection may be either a routine procedure or it may be an operation performed only as an emergency. On the one hand, it may be said to be a prophylactic measure, and on the other a curative measure. We should practice more prophylaxis and not wait until we have trouble at our doors before putting into operation the measures which we expect to help us out of our difficulties.

Most infectious diseases are preventable. Our views concerning the transmission of some of these diseases have already undergone considerable change, and the chances are that before

many years we will have to modify still further what are our present ideas on the subject. At the present time the discrepancies are too great, in some cases at least, between what actually happens under natural conditions and what appears to happen when we substitute artificial conditions for the natural, in our studies of these diseases in the laboratory.

There is no getting away from the fact that frequent and thorough disinfection of a dairy barn would add to the gross cost of producing milk. Moreover, the results of any system of disinfection are not readily apparent from day to day. For these reasons the average dairyman is naturally dubious about going to the additional expense. However, to those who have given the matter careful thought, it appears to be good business to not stint the use of disinfectants. Intelligent stable management means fewer sick cows, and consequently fewer dead ones. Sick cows are usually non-producers and dead cows have to be replaced. In either event there is an actual increment to the cost of producing a pound of milk. Healthy cows will keep this steadily increasing figure down to somewhere near where it belongs. Cleanliness of the stables is usually reflected very clearly in the health of the animals confined therein.

Now, as to the best method of disinfecting a stable, only a few general rules can be given here, because what would apply to one case might very likely fail to apply in the next.

When possible, all animals should be removed from the barn while the disinfection is in progress. All litter should be removed from the stalls, and if the situation warrants the procedure, this litter should be burned as near to the barn as safety will permit. The shorter the distance the litter has to be transported the better. Perhaps the most efficient way of applying any disinfectant in liquid form is by means of a power sprayer, or spray pump, similar to those used for spraying fruit trees. For the daily disinfection of floors, alleys and runways, a knapsack sprayer is very convenient.

When the work of disinfection is left to the help around the barn, care should be taken to see that proper instructions are issued, especially as to the dilution of the disinfectant. If this is done in a "hit-or-miss" fashion, the solution will be either too weak or too strong. In most cases these dilutions have been care-

fully worked out and the directions on the label on the container should be followed very carefully, both for the sake of economy as well as efficiency. The most efficient disinfectant may be wasted and its effect lost by a careless operator who fails to follow directions.

Your president suggested that I say a word concerning milking machines, from the bacteriological standpoint. The use of these machines seems to be growing in favor in modern dairies. At least two reasons for this are readily apparent, the increasing scarcity of farm labor and the possibilities in the way of producing a cleaner milk. However, the latter may not always work out as we would wish it, as shown by Ruediger. The latter showed that the milking machine may actually be a factor in the production of milk that was dirty, from the bacteriological standpoint. Instead of keeping the milk free from contamination, just the opposite may happen unless the teat cups and rubber tubing are very carefully cleaned and scalded before each milking. Chemical disinfectants are generally unsuitable for milking machines and their attachments.

One other word, and that is concerning the disposal of manure. Certain dangers in the way of infectious and parasitic diseases may lurk in stable manure. These dangers may be kept at a minimum by the prompt and proper removal of the manure from the barn, and then seeing that it is properly disposed of. It is considered good farm management to haul the manure directly to the fields. When this is done and the manure subsequently plowed under, the chance for damage from any bacteria or parasite eggs is slim indeed. Likewise, manure that is properly composted, probably automatically disinfects itself. This comes about through the production of heat incident to fermentation. The free use of a good disinfectant in the gutters behind the cows will do much toward minimizing the dangers from infected manure in stables where an infectious disease is known to exist. The use of chlorinated lime or freshly slaked lime has met with considerable favor in this connection. Lime in the form of whitewash has some good points in its favor. The disinfectant powers of the lime may be augmented by the addition of some other disinfectant just before the whitewash is applied.

A little stunt that may repay the effort many times is to have

a shallow tray of some disinfectant solution located near the door of the barn. This is especially true if the barn is one that is frequently visited by strangers, or if an infectious disease is known to exist in the neighborhood. Everyone entering the barn should be instructed to disinfect his shoes. The practice may also have very desirable psychological value.

A recent publication of the Department of Agriculture suggested the use of bichloride of mercury as a disinfectant in preference to carbolic acid or any of the coal-tar preparations, where the odors of these would be objectionable, due to the ease with which milk and milk products absorb odors. Just a word of warning in this connection. If the stable has iron floor drains, traps or sewer pipes, the bichloride of mercury will corrode these in time, even when weak solutions are used. Tile drains would not be so affected.

Skinning of the carcasses of dead animals is a common practice for the purpose of saving and selling the hides. This should not be done unless the cause of death is determined, and it is known that no infection may be spread through the agency of the hide. The latter may be made safe by immersion in a bath of disinfectant for twenty-four hours, except in the case of anthrax, where a special procedure is necessary in order to kill any spores that may be present. However, it is a very dangerous practice to save a hide from an animal that has died of anthrax and should never be attempted.

The prevention of certain animal diseases has been made possible by vaccination, but it was never the intention of our scientists to have vaccination replace sanitation.

Studies from the Research Laboratory.

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THE ANTHELMINTIC TREATMENT OF EQUINE INTES- TINAL STRONGYLIDOSIS.

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Strongyles, including for the most part species of the genus *Strongylus* (*Sclerostomum*) and *Cylicostomum* (*Trichonema*, *Cylicostomum*, "*Sclerostomum tetracanthum*"), are very common parasites of the large intestine of the horse, and are regarded as rather serious parasites. The adult worms of the genus *Strongylus* are blood-suckers, as their red color indicates, and the habit of sucking blood produces here, as elsewhere, resultant anemic conditions and the associated lowering of vitality and of resistance to other injurious factors. The larval worms develop in various organs and tissues outside of the digestive tract and in the walls of the digestive tract, acting as foreign bodies and occasioning varying degrees of injury according to location. The larvæ of *Strongylus vulgaris* cause aneurisms of the great mesenteric artery, and later pass, as agamic adults to the walls of the cecum, where they form small cysts or abscesses. The larvæ of *Str. equinus* usually occur in the liver, lungs and pancreas. The larvæ of *Str. edentatus* are especially apt to occur under the serous membranes, the peritoneum and pleura, but may occur almost anywhere. The larvæ of *Cylicostomum* occur in cysts in the walls of the large intestine. Verminous aneurisms are well known to veterinarians as the potential cause of sudden death by rupture, of intermittent lameness from embolism due to particles from the aneurism lodging in the blood vessels of the hind legs, and of verminous colic from embolism similarly occasioned occurring in the blood supply of the large intestine.

The symptoms resulting from infestation with these worms

are diarrhoea, loss of appetite, emaciation, and anemia. Later the animal may show edema, joint infection, intermittent colic, or the other symptoms noted. The condition, like almost all worm diseases, is afebrile. The disease may prove fatal, become chronic as the result of injuries to tissues, or the animal may recover.

The noted French authority, Railliet (1915), states that it is difficult to expel parasites from the large intestine by oral medication in any host species, and this is the general view of parasitologists and veterinarians. It is quite decidedly the prevailing view as regards the expulsion of strongyles from the horse. Here the worms are remote from the mouth and associated with large masses of undigested material in a way that makes dilution of the anthelmintic certain and contact with the worms theoretically difficult. Somewhat to our surprise, our experiments showed that these worms could be removed with great certainty and with a high degree of efficacy as regards the number removed compared with the total number present. Inasmuch as our findings in these experiments are completed by postmortem examination, these results are dependable. Of course, occasionally failures must be expected, but strongylidosis is more susceptible of successful treatment than has been thought.

Our explanation of the high degree of efficacy obtained in the removal of worms from the cecum and colon of the horse is that it is due to the increase in the time factor, as regards period of exposure of the worm to the anthelmintic. Anthelmintic efficacy is a product of certain factors—the potency of the drug, the amount of the drug, the contact with the worms, and the period of contact with the worms. In a general way, an increase in any of these factors increases their product, which is the anthelmintic efficacy. Food and drugs pass rather rapidly from the stomach and through the small intestine of the horse, but they lie for comparatively long periods in the cecum and double colon. It seems entirely probable that the long period in which anthelmintics may operate in the large intestine is responsible for the high efficacy they attain. In this connection it should be noted that food enters the cecum of the horse through one aperture and passes out through another, a condition not present in such animals as the chicken, dog, swine, sheep, cattle, etc. In the latter

animals, it is theoretically possible, and, in the case of the dog, entirely probable from the evidence of such anthelmintic experiments as those of Hall and Foster (1918), for drugs to pass the ileocolic or ileocecal valve to the colon without entering the cecum. This is not possible in the horse, so that drugs can be depended on to enter the cecum if they pass the ileocecal valve.

The method used by us in our experiments was the one already published by one of us (Hall, 1917) in connection with a study of the action of carbon bisulphide on bots. One of us (Wilson) administered the drugs, supervised the feeding and fasting of the horses and the collection of the manure and made the general postmortem examination. The others made daily collections and identifications of the worms from the manure, collected and identified the worms present postmortem, and noted the condition of the digestive tract postmortem. Worms were only identified as far as their genus, it being out of the question to identify species of *Cylicostomum* *Trichonema* from manure in work of this sort and unnecessary to do this or identify species of *Strongylus* in ascertaining efficacy. Our results do not indicate that any particular species shows any special resistance. The manure was examined daily, being picked apart slowly and carefully. This is a slow and tedious task, but entirely feasible. A few *Cylicostomum* are doubtless overlooked, but the oversights antemortem and postmortem probably offset one another, leaving the ascertained percentage of anthelmintic efficacy substantially correct. Rubber gloves were worn to protect the hands, but the task of examining manure thus is not so unpleasant as might be imagined. On postmortem examination, the contents of the large intestine were examined in the same way, and the worms collected, counted and identified. Our work covered the efficacy of our drugs against other worms, as well as strongyles, and these findings are covered in the paper immediately following this.

Railliet (1915) notes that Giles gave a pony thymol to remove *Cylicostomum*, using 3 doses of 15 grams each, which removed many worms. Subsequently he gave the same animal a lavage with a watery emulsion of 45 grams of thymol dissolved in alcohol. This killed the worms and the horse. Railliet also notes that Theobald gave a horse thymol, 1 gram in the morning and 1 gram in the evening, the dose being dissolved in 30 grams of

alcohol. The next day he gave castor oil. Theobald claims that this killed strongyles, ascarids and pinworms, and even killed the encysted forms. Railliet further notes that Dorn and Bochsberg used atoxyl, the former injecting 3 grams in 100 grams of water at 37°C., and the latter injecting 0.2 to 1.5 grams in 1 per cent saline solution intravenously and subcutaneously. Leneveu (1915) recommends the use of carbon bisulphide in gelatine capsules, giving 2 to 5 grams, according to the size of the animal, every day for 5 days, and following this on the sixth day with a purgative, preferably magnesium sulphate. Conreur (1915) gives 1- to 2-year-old colts a hard soap bolus containing 6 gms. of thymol, a half a gram of santonin, and 6 gms. of aloes. One bolus is given every 2 to 4 days for a total of 3 or 4 doses. The dose is doubled for a 3-year-old.

In our experiments, some of the common anthelmintics which are given to horses for worms, usually for ascarids, were tested. These anthelmintics were iron sulphate, tartar emetic, and turpentine. In addition we tested oil of chenopodium, which has been recommended for worms in horses by Thum (1915) and by Woolridge (1916). Thum gives suckling foals 3 doses at 2-hour intervals for a total of 50 drops, followed 2 hours later by castor oil if desired, and gives 50 to 100 drops to colts which are weaned. He thinks it is much safer than tartar emetic. Woolridge gave a horse 1 dram of oil of chenopodium and 40 grains of thymol twice a day for a month and reports that the animal passed myriads of worms and became fat. The dosage used and recommended for the other drugs named varies considerably. Iron sulphate is given in doses of 1 ounce to the fasting animal, in 2- to 4-dram doses in a mash, twice a day for 7 days. Tartar emetic is given in doses of one ounce in aloes ball to the fasting animal, 2 to 4 drams in a mash twice a day for 5 days, etc. Turpentine is usually given in doses of 1 to 4 ounces in a half pint to a quart of linseed oil. Place (1915) says of turpentine: "One or two teaspoons of chloroform increases the effectiveness of the mixture and the risk."

Our experiments were as follows:

Horse No. 1640, a 14-year-old gelding weighing 1075 pounds, was given 2 drams of iron sulphate in a mash daily for 7 days. The third day of treatment the horse passed 2 *Cylicostomum*, the

fourth day 1, and the sixth day 1, a total of 4 *Cylicostomum*. The horse was killed 10 days after the last treatment, the manure being examined during this period following treatment. On postmortem examination the animal had 288 *Cylicostomum* and 80 *Strongylus*. The treatment was 0 per cent effective against *Strongylus* and much less than 1 per cent effective against *Cylicostomum*; in other words, a failure.

Horse No. 32, an 11-year-old gelding weighing 1250 pounds, was given 2 drams of tartar emetic in a mash daily for 5 days. The third day the horse passed 1 *Cylicostomum*, the fourth day 1, the first day after the last treatment 2, the third day 4 *Cylicostomum* and 1 *Strongylus*, the seventh day 1 *Cylicostomum*, and the twelfth day 1, a total of 10 *Cylicostomum* and 1 *Strongylus*. On postmortem the horse had 5474 *Cylicostomum* and 312 *Strongylus*. The treatment was therefore less than 1 per cent effective against *Cylicostomum* and *Strongylus*; in other words, a failure. The small intestine showed numerous petechiæ and ecchymoses which were apparently due to the action of the drug.

Horse No. 371, a 9-year-old gelding weighing 1050 pounds, was given 2 ounces of turpentine, followed immediately by a quart of linseed oil. The next day the horse passed 9 *Strongylus* and 56 *Cylicostomum*, the second day 50 *Strongylus* and 211 *Cylicostomum*, the third day 18 *Strongylus* and 3 *Cylicostomum*, the fourth day 3 *Strongylus*, the fifth day 22 *Strongylus*, the sixth day 3 *Cylicostomum*, a total of 102 *Strongylus* and 274 *Cylicostomum*. The seventh day the animal passed no worms and was killed. On postmortem examination the horse had 105 *Strongylus* in the cecum and 7 in the colon, a total of 112; no *Cylicostomum* was found. The treatment was therefore 100 per cent effective against *Cylicostomum* and 48 per cent effective against *Strongylus*, a very good showing. This horse had been fasted less than 24 hours, and it is possible that greater efficacy would have resulted from a longer period of fasting.

Horse No. 1641, a 13-year-old mare weighing 1100 pounds, was given 8 mls of oil of chenopodium, followed immediately by a quart of linseed oil. The third day after treatment the horse passed 1 *Cylicostomum*, the fourth day 1 *Cylicostomum*, and the sixth day 1 *Strongylus* and 430 *Cylicostomum*. The treatment was therefore less than 1 per cent effective against strongyles; in

other words, a failure. This horse had been fasted less than 24 hours.

Horse No. 89, an 11-year-old gelding weighing 1070 pounds, was given 10 mls of chenopodium, a somewhat larger dose than in the previous case, followed immediately by a quart of linseed oil. The second day the horse passed 5 *Strongylus* and 169 *Cylicostomum*, and the third day 2 *Cylicostomum*. The horse was killed the third day. On postmortem examination, 16 *Cylicostomum* were found dead and being passed out in the floating colon, making a total of 187 *Cylicostomum* to be credited to the anthelmintic. There were still left 1545 *Strongylus* and 448 *Cylicostomum*. The treatment was therefore less than 1 per cent effective against *Strongylus* and was 29 per cent effective against *Cylicostomum*. The horse had been fasted less than 24 hours and was inadvertently fed shortly before treatment.

Horse No. 272, an 11-year-old gelding weighing 1150 pounds, was given 16 mls of chenopodium, double the dose given to No. 1641, followed immediately by a quart of linseed oil. The next day the horse passed 4 *Strongylus* and 17 *Cylicostomum*, the second day 7 *Strongylus* and 15 *Cylicostomum*, the third day 39 *Strongylus* and 70 *Cylicostomum*. The horse was killed on the fourth day and found to have 19 *Strongylus*. The treatment was therefore 100 per cent effective against *Cylicostomum* and 76 per cent effective against *Strongylus*. The horse was fasted less than 24 hours before treatment.

Horse No. 273, an 11-year-old gelding weighing 1100 pounds, was given 18 mls of chenopodium, followed immediately by a quart of linseed oil. The next day the horse passed 64 *Cylicostomum*, the second day 293 *Cylicostomum* and 7 *Strongylus*, and the third day 64 *Cylicostomum* and 1 *Strongylus*, a total of 421 *Cylicostomum* and 8 *Strongylus*. On postmortem examination the horse had 7 *Cylicostomum* and 1 *Strongylus* in the floating colon, which should be credited to the efficacy of the anthelmintic. There were also 102 *Strongylus* and 3195 *Cylicostomum*. The treatment was therefore 11 per cent effective against *Cylicostomum* and less than 1 per cent effective against *Strongylus*. The horse was fasted less than 24 hours before treatment.

Horse No. 1033, a 6 year old gelding weighing 1075 pounds, was given 16 mls of chenopodium, followed immediately by a

quart of linseed oil, the horse having been fasted a full 24 hours before treatment. The next day the animal passed 1 *Cylicostomum*, the third day 30 *Cylicostomum* and 30 *Strongylus*, the fourth day 34 *Cylicostomum* and 49 *Strongylus*, and the fifth day 12 *Cylicostomum* and 8 *Strongylus*, a total of 77 *Cylicostomum* and 107 *Strongylus*. The animal was killed on the fifth day. On postmortem examination there were found 2 larval *Cylicostomum* that might have issued from a cyst in the esophageal mucosa after the treatment, and probably did do this. Regarding them as having issued from their cysts after the passage of the anthelmintic, the treatment was 100 per cent effective against *Cylicostomum* and *Strongylus*. Even regarding them as surviving the anthelmintic would make the treatment 97 per cent effective against *Cylicostomum*.

Horse No. 240, an 8-year-old gelding weighing 1100 pounds, was given 16 mls of oil of chenopodium followed 2 hours later by a quart of linseed oil. The next day the horse passed 352 *Cylicostomum* and 1 *Strongylus*, the second day 184 *Cylicostomum* and 26 *Strongylus*, the third day 4 *Cylicostomum* and 22 *Strongylus*, the fourth day 6 *Strongylus*, and the fifth day 2 *Strongylus*, a total of 540 *Cylicostomum* and 61 *Strongylus*. The horse was killed on the fifth day. On postmortem examination, 2 dead *Strongylus* were found in the floating colon and 3 dead *Strongylus* in the double colon, which worms must be regarded as killed by the anthelmintic. There were also 3 live *Strongylus* in the cecum. The treatment was therefore 100 per cent effective against *Cylicostomum* and 96 per cent effective against *Strongylus*.

Horse No. 1031, an 8-year-old gelding weighing 1060 pounds, was given the iron sulphate treatment. The intention was to give doses of 4 grams of iron sulphate twice daily in a mash, for a period of 7 days, but as the horse refused to clean up this amount of medicated mash, the 14 doses were administered over a period of 12 days. The manure was only casually examined for *Strongylus* and *Cylicostomum*, being primarily examined for ascarids. The third day of the treatment the horse passed 1 *Cylicostomum*, the fifth day 2 *Cylicostomum*, a total of 3 *Cylicostomum*. Sixteen days after beginning treatment, the horse was given 3 doses of 6 mls of chenopodium at hour intervals, the last

dose being followed an hour later by a quart of linseed oil. The horse was fasted over 24 hours. The day of treatment the horse passed 49 *Cylicostomum*, the following day 1024 *Cylicostomum* and 54 *Strongylus*, the second day 103 *Cylicostomum* and 11 *Strongylus*, the third day 30 *Cylicostomum*, the fourth day 35 *Cylicostomum* and 6 *Strongylus*, and the fifth day 1 *Cylicostomum* and 5 *Strongylus*, a total of 1242 *Cylicostomum* and 76 *Strongylus*. The horse was killed on this fifth day and found to have 2 larval *Cylicostomum*, which we regard as having left their cysts in the intestinal mucosa after the anthelmintic had passed out, and 4 live *Strongylus* in addition to the 2 dead *Strongylus* passing out in the floating colon. The treatment was therefore 100 per cent effective against *Cylicostomum* and 95 per cent effective against *Strongylus*.

From the foregoing experiments we may come to the following conclusions:

Iron sulphate in the light dose used (2 drams in a mash daily for 7 days) was a failure, removing no *Strongylus* and less than 1 per cent of the *Cylicostomum* present. Not too much may be concluded in regard to the value of larger doses, but in view of the fact that this treatment is not recommended for strongyles, it is likely that it is not of much value. This conclusion is substantiated by the poor results obtained from the administration of 7 ounces of iron sulphate over a period of 12 days in the case of Horse No. 1031.

Tartar emetic in the light dose used (2 drams in a mash daily for 5 days) was a failure, removing less than 1 per cent of the strongyles present. The evidence of severe irritation in the digestive tract postmortem inclines us to believe that this drug is not apt to prove of much value in this condition, as increased size of dose to secure greater efficacy would mean a degree of gastro-intestinal irritation that in our opinion should be avoided.

Turpentine in a moderate dose (2 ounces in a quart of linseed oil) was a rather effective remedy in the one test made, removing all of the *Cylicostomum* and 48 per cent of the *Strongylus*.

Oil of chenopodium was a failure in small doses with less than a 24-hour fast, failing to remove 1 per cent of the strongyles present in a dose of 8 mils; it was less than 1 per cent effective against *Strongylus* and only 29 per cent effective against *Cylicos-*

tomum in a dose of 10 mils. In larger doses, with less than a 24-hour fast before treatment, the findings are somewhat contradictory: a 16-mil dose was 100 per cent effective against *Cylicostomum* and 76 per cent effective against *Strongylus*, while an 18-mil dose was 11 percent effective against *Cylicostomum* and less than 1 percent effective against *Strongylus*. In these same larger doses, with fasts of at least 24 hours, the treatment is highly effective. In one case, where the chenopodium and linseed oil were given simultaneously, the treatment was apparently 100 per cent effective against strongyles; in another case, where the linseed oil was given 2 hours after the chenopodium, the treatment was 100 per cent effective against *Cylicostomum* and 96 per cent effective against strongyles; in another case, where the chenopodium was given in divided doses followed by linseed oil an hour after the last dose, the treatment was 100 percent effective against *Cylicostomum* and 95 percent effective against *Strongylus*.

SUMMARY.

Contrary to what has been supposed, the removal of strongyles from the large intestine of the horse presents no great difficulties. The remedy of choice is oil of chenopodium, which displays an efficacy of 95 to 100 per cent when given to horses fasted 36 hours and given in doses of 16 to 18 mils, in one dose or in divided doses, accompanied by a quart or a liter of linseed oil or followed one or two hours later by this amount of linseed oil. The small worms, *Cylicostomum*, are more readily removed than the large, red palisade worms, *Strongylus*, probably due to the fact that *Strongylus* attaches to the mucosa and *Cylicostomum* does not. Turpentine appears to be the second choice of the remedies tested. In the doses used, iron sulphate and tartar emetic gave very poor results and promised little of value in the treatment of strongylidosis.

BIBLIOGRAPHY.

- CONREUR, CHARLES, 1915. Arch. Brazil Med., v. 5 (8) Aug., pp. 323-348.
 HALL, MAURICE C., 1917. Jour. A. V. M. A., n. s., v. 5 (2), Nov., pp. 177-184.
 HALL, MAURICE C., and WINTHROP D. FOSTER, 1918. Jour. Agric. Research, v. 12 (7) Feb. 18, pp. 397-447, 1 fig.
 LENEVEU, G., 1915. Rev. gen. d. méd. vét., Toulouse, v. 24 (228), Dec. 15, pp. 593-612.
 PLACE, 1915. Jour. Agric. S. Afric., May.
 RAILLIET, A., 1915. Rec. d. méd. vét., Par., v. 19 (15), Aug. 15, pp. 490-513.
 THUM, H., 1915. Zeits. f. Tiermed., n. f., v. 81 (11-12), pp. 503-528.
 WOOLRIDGE, PROF., 1916. Vet. News, v. 13 (638), Mar. 25, pp. 125-126.

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SOME NOTES ON THE TREATMENT OF EQUINE ASCARIASIS AND OXYURIASIS.

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In the foregoing paper the writers have shown that equine intestinal strongylidosis, contrary to what might be supposed, is a disease readily amenable to treatment so far as the removal of the adult worms from the intestine is concerned. In this paper we can confirm the idea that equine oxyuriasis is readily amenable to anthelmintic treatment, as has been stated by such authorities as Railliet, and the idea that equine ascariasis is not readily amenable to anthelmintic treatment by therapeutic doses of safe anthelmintics of which we are at present aware, as Neveu-Lemaire has noted.

In the series of 10 horses used in the anthelmintic investigations reported in our foregoing paper, 7 had infestations with *Oxyuris equi*. The anthelmintic treatments given (for which, see the paper referred to) removed 100 per cent of the pinworms present from 5 of the 7 horses, as follows: Horse No. 240, 2 worms; Horse No. 1031, 1 worm; Horse No. 1033, 1 worm; Horse No. 32, 1 worm; Horse No. 371, 34 worms. Treatments failed entirely to remove worms and left worms present as follows: Horse No. 273, 2 worms; Horse No. 1640, 3 worms.

Reference to the foregoing paper shows that 18 mls of oil of chenopodium, followed immediately by a quart of linseed oil, in the case of an animal that had fasted less than 24 hours, was a failure, and that 2 drams of iron sulphate in the feed daily for 7 days was a failure. It may be noted also that the administration of 7 ounces of iron sulphate over a period of 12 days to Horse No. 1031, previous to the administration of the chenopodium,

was also a failure. On the other hand, the treatments with adequate doses of oil of chenopodium, 16-18 mls, to animals fasted over 24 hours, with 2-ounce doses of turpentine, and with daily administration of 2 drams of tartar emetic in the feed for 5 days, were entirely successful.

Of the same 10 horses, 8 had ascarids, *Ascaris equorum*. The anthelmintic treatments given were entire failures in the case of 4 of the 8 horses and left worms present as follows: Horse No. 89, 30 worms; Horse No. 371, 15 worms; Horse No. 272, 1 worm; Horse No. 1641, 4 worms. The treatment removed 3 per cent of the worms from Horse No. 1033, removing 7 and leaving 214; 8 per cent from Horse No. 32, removing 1 and leaving 11; 12 per cent from Horse No. 273, removing 2 and leaving 14; and 25 per cent from Horse No. 1031, removing 1 and leaving 3.

The foregoing results are not of themselves especially encouraging, but they constitute a guide for further work, and considering the lack of dependable experimental work in this field they cannot be regarded as discouraging. Equally unsatisfactory results in initial experiments are very commonly followed by entirely satisfactory results, and it is worth while to know what methods will not prove profitable. Modifications in the size of dose or mode of administration of some of the drugs noted may give much higher values for the drugs used. At present we can only state that additional experimental work is necessary before we can feel that we have a dependable anthelmintic for the removal of ascarids from horses.

**Studies from the Research Laboratory.
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THE OCCURRENCE OF TAPEWORMS, ANOPOLOCEPHALA SPP., OF THE HORSE IN THE UNITED STATES.

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One of us (Hoskins) has recently collected 37 specimens of *Anoplocephala mamillana* from the duodenum of one horse (No. 1019) and a single specimen of the same tapeworm from another horse (No. 686), at Parkedale Farm, Rochester, Michigan. The first horse was a 5-year-old bay mare, weighing 1075 pounds, that had been purchased at Chelsea, Michigan, in May, 1917, and had been at Parkedale Farm for almost 11 months. The second horse, a 6-year-old brown mare, weighing 1050 pounds, had been purchased at the same place in August, 1916, and had been at Parkedale Farm almost 21 months. So far as we are aware, from personal experience and from investigation of the literature readily available, this is the first published record of the occurrence of this tapeworm in the United States except for some casual statements. Kinsley (1910) says *Anoplocephala mamillana* has been reported, but we have not found the reports. Dimock (1917) makes a very casual reference to the occurrence of *Anoplocephala mamillana*; it is quite possible that he had cases of *Anoplocephala mamillana*, but his statement is somewhat indefinite.

The fact that no one has taken the trouble to compile the records of the occurrence of horse tapeworms in this country makes it difficult to correlate our record with the records scattered through the literature, and in our opinion warrants an attempt to compile, at least tentatively, the existing records. We have accordingly got together such records as could be readily found, and offer this as a nucleus about which to group new records or those we may have failed to find.

There are three species of tape-worm in the horse, *Anoplo-*

cephala magna (*Anoplocephala plicata*), *Anoplocephala perfoliata*, and *Anoplocephala mamillana*. We have given below a brief description of these worms, with their records from this country and some incidental notations.

Anoplocephala mamillana (Mehlis, 1831) R. Blanchard, 1891.

This is the smallest of the three species, with a total length of 0.6 to 5 cm. Head small, 0.5 by 0.8 mm. in diameter, rather quadrangular, and at times retracted into the anterior end of the strobila. The suckers and their slit-like apertures are elongated parallel to the longitudinal axis of the strobila. There are 30 to 50 segments, which very soon become wider than the small head and attain a maximum width which is maintained to the posterior end of the strobila. Terminal segments may be half as long as wide. Eggs 88 μ long by 50 to 66 μ wide. As many as 72 specimens of this worm have been recorded from one horse. The worms occur in the jejunum and ileum, as a rule, and are sometimes found in the stomach. They are not believed to have much pathological significance. According to Railliet, this species is the commonest of the horse tapeworm at Paris. Neveu-Lemaire (1912) states that teniasis is rare in horses in France, but common in Germany and Russia.

American records: The only definite records of which we are aware are the ones published in this paper. Stiles (1896) has some excellent figures, but his specimens were collected in London by Dr. Albert Hassall. These specimens are also reported by Stiles and Hassall (1894).

Anoplocephala perfoliata (Goeze, 1782) E. Blanchard, 1848.

This species is intermediate in size between *Anoplocephala magna*; specimens are usually 0.8 to 2.5 cm. long, but may attain a length of 8 cm. It is characterized by the peculiar structure of the suckers, which are prolonged posteriorly in 4 rounded lobes. The head is large, 2 to 3 mm. in diameter. There is no neck. The segments very soon become wider than the head, attain a maximum width near the middle of the worm, and gradually narrow posteriorly from the middle. The last segments are often sterile. Eggs 65 by 80 μ in diameter. The worm is usually present in large numbers, even hundreds, and in one case a basketful is said to have been removed from a horse which had become paralyzed and anemic. The worm occurs in the cecum

and ileum, and at times in the colon (the cecum and colon are quite unusual sites for tapeworms of mammals), and is credited with causing pathological conditions, sometimes severe. A recent record of the sort is that of Ferreira and Horta (1917), who report from Lisbon, Portugal, an extensive rupture of the proximal part of the floating colon in a 2-year-old horse, the area adjoining the rupture being inflamed and ecchymotic and the rupture aperture and the abdominal cavity containing a large number of specimens of *Anoplocephala perfoliata*, which they regard as partly responsible, at least, for the pathological condition.

American records. We do not recall or find any records of this parasite from this continent. Stiles and Hassall (1894) report specimens of this worm in the collection of the U. S. Bureau of Animal Industry, but these specimens, like those of *Anoplocephala mamillana*, were collected by Hassall in London. Koon (1913) reports tapeworms from the small intestine under the title of "*Tania perfoliata* a plenty," but we regard these worms from the small intestine as probably *Anoplocephala magna*; the title of the article may have been supplied in a casual way.

Anoplocephala magna (Abildgaard, 1789) Spengel, 1905.

This is a large worm, 9 to 80 cm. long. The head is very large, 4 to 6 mm. in diameter, with cup-like suckers, directed forward, and with grooves around the head posterior of the suckers and at right angles to the long axis of the strobila. There is no neck. The segments increase in width until considerably larger than the head and then usually diminish posteriorly. The eggs are 50 to 60 μ in diameter. This is said to be the rarest of the tapeworms of the horse in Europe. So far as we have information, exactly the opposite is the case here, as this is apparently the commonest of the tapeworm species in the American horse. This worm has been supposed to give rise to intestinal disturbances or anemia at times. It occurs usually in the small intestine, rarely in the stomach.

American records. This worm was reported by Stiles and Hassall (1894) as represented in Leidy's collection by material collected in Philadelphia by Zuill. Kinsley (1910) reports the occurrence of two specimens of this worm, under the name of *Tania plicata*, from a colt in St. Clair county, Missouri, and gives

an excellent photograph of the worms. He states: "Infestations with the *Tænia perfoliata* and *mamillana* have been reported from some localities in the United States, but the writer has been unable to find any authentic report of the observation of tæniasis produced by infestation with the *Tænia plicata* in America." Maxfield (1915) has reported the occurrence of large numbers of these tapeworms from a horse at Tama, Ia., and later (Maxfield, 1917) reported collecting a half bushel of "tapeworms" (possibly *Anoplocephala magna*) from another horse at this place, the worms being apparently from the small intestine. Koon (1913) states that he collected a gallon pail full of tapeworms from the small intestine of a 4-year-old horse at Kingsley, Iowa. The title identifies these as *Tænia perfoliata*, but the site of infestation indicates that they were probably *Anoplocephala magna*. Kinsley (1915) has also reported Maxfield's case (as *T. plicata*=*Anoplocephala magna*), and another case where a colt at Davenport, Nebraska, was found to have 32 specimens of this worm. Kinsley states that these last two cases are the third and fourth reported to him that year, so that two other cases must be added to his detailed records. Some of Kinsley's specimens were examined by one of us (Hall) in the Bureau of Animal Industry, and at that time other specimens already in the collection of the Bureau of Animal Industry were examined and found to consist of material from Ames, Iowa, and Madison, Wisconsin. A year later, Kinsley (1916) reports *Tænia plicata* from the horse at Sabetha, Missouri, noting that the collector, Dr. Edward G. Lahr, "removed about three gallons of them from the large intestines of a horse." This is an enormous amount of tapeworms. The site of infestation, the large intestine, is one from which this tapeworm has never been reported to our knowledge, and casts a certain doubt on the accuracy of the identification. It is regrettable that such an unusual occurrence was not reported with more detail. In comment, Kinsley states: "These worms are reported in veterinary literature to be rare, but a number of alumni have reported them this year." This covers an indefinite number of cases, that cannot be readily incorporated in a summary. Stromlund (1918) has reported 3 tapeworms, 2 to 3 inches long (probably *Anoplocephala magna*) from a foal at Sloan, Iowa.

It appears then that this worm has been found in the United States in over 12 cases from Pennsylvania, Missouri, Iowa, Nebraska and Wisconsin. Stewart (1917) refers to the occurrence of the worm in Southern Texas, but we do not find a reference to cases. In all probability, there are other records that have not come to our attention.

The life history is not known for any of the tapeworms of the horse.

The tapeworms of the horse can be identified by the use of the following key:

Key to the tapeworms of the horse.

1. Head large, 4 to 6 mm. in diameter. Entire strobila commonly not more than 8 cm. long. In small intestine and stomach, *Anoplocephala magna*.

Head not over 3 mm. in diameter. Entire strobila not over 8 cm. long; usually not over 5 cm. long, 2.

2. Head 2 to 3 mm. in diameter and with the suckers prolonged posteriorly in rounded lobes. In ileum and large intestine, *Anoplocephala perfoliata*.

Head less than 1 mm. in diameter; suckers and apertures elongated parallel to the long axis of the worm and without posterior lobes. In small intestine and stomach, *Anoplocephala mamillana*.

SUMMARY.

Anoplocephala mamillana, said to be the commonest of the tapeworms of the horse in Europe, is reported from the horse in Michigan, the first definite record from the United States of which we are aware. *Anoplocephala perfoliata* has never been reported, correctly, from the horse in the United States, so far as readily available references show. *Anoplocephala magna*, said to be the most rare of the tapeworms of the horse in Europe, is the commonest of the three species in this country and has been found in more than 12 cases.

BIBLIOGRAPHY.

- DIMOCK, W. W., 1917. (Discussion of Maxfield, 1917.) American Journal Veterinary Medicine, vol. 12 (5), May, pp. 298-299.
- FERREIRA, AGUEDA AND ALVIA HORTA, 1917. *Anoplocephala perfoliata* (?), Rev. d. Med. Vet., Lisboa, reprint, pp. 18, 1 fig.
- KINSTLEY, A. T., 1910. Equine teniasis. Kans. City Vet. Coll. Quart., Bull. 28, June, pp. 682-683, 1 pl., 1 fig.
1915. *Tenia plicata*. Kans. City Vet. Coll. Quart., Bull. 48, June, pp. 1172-1173, 2 figs.
1915. *Anoplocephala magna* (*Tenia plicata*). Kans. City Vet. Coll. Quart., Bull. 48, June, p. 1173.
1916. More *Tenia plicata*. Kans. City Vet. Coll. Quart., Bull. 54, Dec., p. 1315.
- KOON, C. H., 1913. *Tenia perfoliata* a plenty. Am. J. Vet. M., v. 8 (4), Apr., p. 234.
- MANFIELD, FRED M., 1915. Teniasis in a horse. Am. J. Vet. M., v. 10 (7), July, p. 495.
1917. Common parasites of the digestive tract. Am. J. Vet. M., v. 12 (5), May, pp. 295-297, 1 fig.
- NEVEU-LEMAIRE, M., 1912. Parasitologie des animaux domestiques. Paris, 1257, pp., 77 figs.
- SHAW, S., 1917. (Discussion of Maxfield, 1917.) Am. J. Vet. M., v. 12 (5), May, p. 298.
- STILES, CH. WARDELL, 1896. A revision of the adult tapeworms of hares and rabbits. Proc. U. S. Nat. Mus., (1105), v. 19, pp. 145-235, pls. 5-25.
- STILES, CH. WARDELL, AND ALBERT HASSALL, 1894. A preliminary catalogue of the parasites contained in the collections of the United States Bureau of Animal Industry, United States Army Medical Museum, Biological Department of the University of Pennsylvania (Coll. Leidy) and in Coll. Stiles and Coll. Hassall. Vet. Mag., v. 1 (4), Apr., pp. 245-253; (5), May, pp. 331-354.
- STROMLUND, E. V., 1918. Intestinal parasites in a foal. Am. J. Vet. M., v. 13 (1), Jan., pp. 14-15.

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BRAZILIAN JALAP AND SOME ALLIED DRUGS.*

BY OLIVER ATKINS FARWELL.

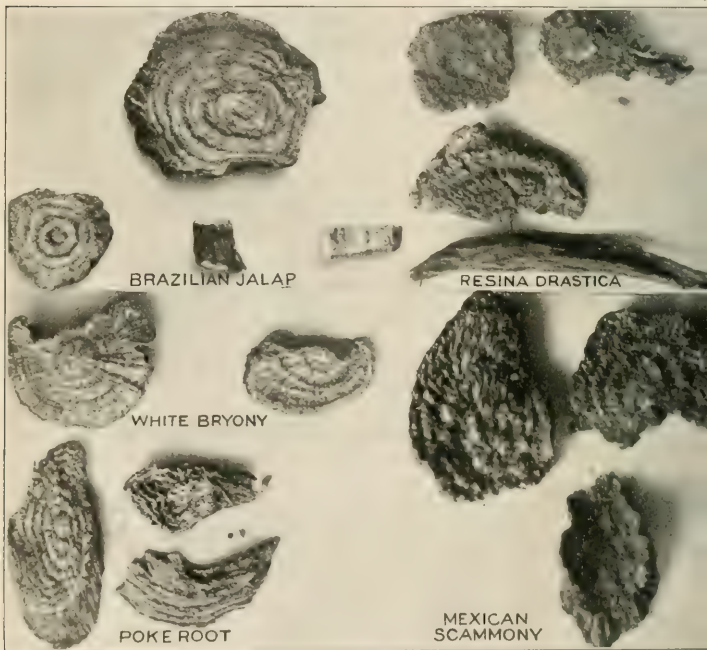
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In the *Pharmaceutical Journal* for November 27, 1915, Mr. E. M. Holmes described a root known as Brazilian Jalap, which he refers to the *Piptostegia Pisonis* Mart. This species was described by Martius in his *Systema Materia Medica Braziliensis*, page 78, in 1843. In addition to this and the typical species, *P. Operculata* Reichb., Martius described and listed *P. Gomesii*. In the *Flora Braziliensis* Meisner reduced the latter to the limbo of synonymy, placing it under *Operculina Convolvulus* but made no mention of *P. Pisonis*; since this so-called species was not mentioned in the *Flora Braziliensis*, the inference to be drawn therefrom is that it was thought to be invalid, just a synonym of *O. Convolvulus*. At different times this species has been included under *Convolvulus* or *Ipomoea*, but at present it is considered to constitute a genus distinct from either, the oldest name for which is *Operculina*. If my deductions as above outlined are correct the proper binomial for the Brazilian Jalap is *Operculina macrocarpa* (Linn.) Urban. The names under which this drug is commonly known in Brazil are *Batata de Purga* and *Batata purgante*. *Tapioco de Purga* is a product derived from the root. The generic characters of *Operculina* are the pear-shaped calyx, large imbricated sepals chartaceous in fruit, broadly campanulate corolla tube, and the twisted anthers. The plant is a climbing shrub with winged stems, palmately 3-7-divided leaves, peduncles about as long as the leaves and white campanulate flowers. Mr. Holmes described the root as follows:

"The root occurs in commerce in the form of transverse circular sections averaging about 1½-2 inches in diameter, and

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about $\frac{1}{4}$ inch in thickness, marked with several concentric rings, and, save for its pale grayish brown tint and the presence of numerous dots of translucent pale resin on the surface, bears considerable resemblance to White Bryony root."



Brazilian Jalap and Some Allied Drugs.

An effort was made to obtain a small supply of the root; in due course of time it was procured from London. It agreed in all points with the description quoted above. We may add, however, that it has a thin dark brown or blackish brown cork, the surface is not fibrous, and that the phloem and cambium are shrunk below the surface of the zylum and cortex; that its re-

semblance to the cross-sections of Mexican Scammony or of Poke is as equally, if not more, pronounced as to that of White Bryony.

The root probably is a large tuberous root and is composed chiefly of soft tissues. The cambium forms complete circles and together with the phloem are, in the commercial drug, noticeable as dark concentric circles shrunk a little below the level of the surrounding tissues. In the sections observed, the tissues were all of secondary growth; that is, spiral or annular tracheæ usually associated with primary meristem could not be detected. The center of the root in the smaller sections is nearly solid xylum, there being four narrow strands of secondary cortex, thus making the structure of the root a tetrarch. The larger sections are very similar but may have less xylum and more cortex. The cambium of the collateral bundles soon ceases its activity and the cortex gives rise to another meristematic region beyond the phloem, and this operation is continued repeatedly, producing in this unusual way its growth in thickness. The xylum forms a very small bundle of few vessels and these are rather widely separated, forming an interrupted circle with wide spaces between the bundles. The medullary ray is usually one cell, sometimes two cells, in width. The vessels of the bundle are reticulated tracheæ, often 0.13 mm. in diameter with walls 0.008 mm. thick, and these constitute the greater portion of the strengthening tissue of the root; tracheides and wood fibers are frequent in the central circles but absent or only occasional in the outer ones. Rosette crystals of calcium oxalate are frequent just internal to the cork, scattered through the cortex, and form crystal fibers close to the xylum. Starch is present only in a very small amount; the grains are simple and range from 0.002 mm. to 0.024 mm. in diameter. Bast fibers are absent or at least were not detected; but most of the bast parenchyma and some of the cells of the medullary rays and secondary cortex, while retaining comparatively thin walls, are stained brown with chloro-iodide-of-zinc, indicating suberization or cutinization. The cells of the cortex are more or less polygonal and average about 0.044 mm. in diameter, the walls are thin and the air space between the cells is very small. The cork layer is about 0.123 mm. thick, and is composed of a series of thin-walled, more or less tabular cells

(0.008 mm. by 0.048 mm.) only the outermost series having thickened cell walls. The tracheides average about 0.44 mm. in length, 0.026 mm. wide, and have a wall about 0.003 mm. in thickness. The wood fibers have oblique pores and range from 0.62–0.78 mm. in length, 0.013–0.020 mm. wide, and have a lumen varying from $1/5-3/4$ the width of the fiber.

RESINA DRASTICA.

The drug that has come to us under this name is of unknown origin. It comes in both transverse and longitudinal sections of the root, the color is a dirty brown or dark grayish brown, much darker than the Brazilian Jalap. In the cross-sections the wood strands project as much as 2 mm. beyond the surface, giving a rough fibrous aspect to the section; the bundles are irregularly scattered through definite concentric zones. In the longitudinal sections the strands appear on the surfaces as smooth ridges in more or less parallel but interrupted lines and frequently extend beyond the end of the section as coarse fibers up to 3 cm. in length and 3 mm. in thickness. From the general resemblance of this root to that of Brazilian Jalap and to that of Mexican Scammony, I would hazard the guess that it is from some plant closely allied to them and consequently from the morning glory family, the *Convolvulaceæ*.

W. L. Scoville, who is working out the chemistry of the drug, has informed me that in so far as he can tell from the limited amount of work he has been enabled to give to it, it does not differ materially from the resin of Mexican Scammony except in its yellow color. Under the microscope the root structure differs from the Brazilian Jalap in having no rosette crystals or crystal fibers, in having a superabundance of starch, the grains being of a more uniform size of from 0.013 mm.–0.018 mm., the vessels being chiefly of the pitted type, wood fibers are plentiful and there is some bast fiber. In Brazilian Jalap, oil with refractive inclusions, perhaps oleoresin, was scarce and where present was arranged in masses, rather than in drops, in longitudinal lines, mostly in connection with the medullary ray cells; but most of the cells of the samples I had for examination were empty, while on the other hand in the *Resina Drastica* samples the cells were well filled with the products of metabolism, and as in the

case of the starch there was a superabundance of large drops of oil with its inclusions throughout the cortex. Wood fibers in this drug measure for the most part from 0.704 mm. to 0.892 mm. in length, about 0.02 to 0.03 mm. wide, the lumen being about $\frac{1}{3}$ the width of the cell.

MEXICAN SCAMMONY.

This drug is derived from the large tuberous roots of *Ipomœa Orizabensis* (Pell) Ledenois and is also known as Male or Orizaba Jalap. It resembles very closely the drug described above as *Resina Drastica*, but is somewhat lighter in color, extremes being as light on one hand as the Brazilian Jalap and as dark on the other as *Resina Drastica*; it is as fibrous as the latter but the strands usually are finer, sometimes longer, more numerous, and arranged more regularly in concentric circles or zones. Under the microscope it differs in no tangible way from the *Resina Drastica*. The resin obtained from this is black; whether or not the difference in the colors of the resins of this and of the *Resina Drastica* can be correlated with specific difference in the plants producing them can not now be determined.

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**OZENA AND DISTEMPER: COMPARATIVE STUDY OF
COCCOBACILLUS FOETIDUS-OZAENAE AND
BACILLUS BRONCHISEPTICUS.**

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(From the Research Laboratory of Parke, Davis & Company, Detroit, Michigan.)

The question of the relationship of ozena to distemper has been frequently discussed, both in print and otherwise. Many observers consider the dog a carrier of an organism common to both diseases; and the patients themselves are often of the same opinion as they will frequently volunteer information bearing on their association with dogs, special emphasis being laid on a co-incident infection of the animal with distemper some time in the early part of the disease.

Perez (1913) carried out some experimental work along this line and suggested that the infection in ozena probably originates from dogs, and, as a result of his work, cultures from these animals were included in an ozena vaccine upon which Hofer and Kofler (1914), collaborators of Perez, were experimenting.

Horn and Victors (1916) in this country, considering *Coccobacillus fœtidus-ozænæ* (Perez) as the principal etiological factor in ozena and *Bacillus bronchisepticus* (Ferry) as the cause of distemper, have even attempted to prove a relationship between the above mentioned organisms and claim to have found a positive complement fixation between them. The question, however, was left in rather an unsettled condition, as may be seen by the following statement made by them:

"*Bacillus bronchisepticus*, exhaustively studied by Ferry, M'Gowan, Torrey and Rahe, and determined by them as being the specific organism of canine distemper, morphologically almost identical and biologically in many instances similar to the Perez organism, and the advanced hypothesis that the infection of fetid ozena is carried by dogs, presents an interesting complex."

It is not the province of this paper to discuss nor to attempt to offer any proof pro or con as to the etiology of distemper in the dog or of ozena in the human subject (that phase of the question having been taken up by the several authors elsewhere); but rather to attempt to answer the question as to the relationship of Perez' organism to *B. bronchisepticus*, which has been so significantly brought forward by Horn and Victors.

If Perez' bacillus is the cause of ozena, and it is demonstrated to be the same as, or closely related to, *B. bronchisepticus*, an extremely important point has been established and its bearing on the prophylaxis and management of ozena can hardly be estimated at the present time, since distemper is most widely distributed among dogs and other small animals, especially laboratory animals, such as the rabbit and guinea-pig. Accordingly, the authors, using several typical strains of both organisms, have carried on a large number of comparative morphological, cultural and serological tests in an attempt to determine, if possible, the characteristics common or perhaps, to be more exact, not common to both organisms.

For the morphological and cultural characteristics of *B. bronchisepticus* those originally published by one of us (Ferry, 1910, 1911) and corroborated by M'Gowan (1911) and Torrey and Rahe (1913) are taken as characteristic; and for *Coccobacillus fatidus-ozenæ* only those described by Ward (1917) are considered, as they apply directly to the strains under discussion in this work. Unfortunately, there does not seem to be a unanimity of opinion among writers relative to all reactions of this organism. The question of motility seems to be still undecided. The four strains under discussion, however, were found by the authors to be motile, although not so progressively active as *B. bronchisepticus*. In this instance the authors do not agree with Ward, who claims that the European strains are non-motile.

SUMMARY OF CULTURAL REACTIONS.

1. *B. ozenæ* may be readily differentiated from *B. bronchisepticus* by any one of the following reactions: by growth on potato, on Loeffler's blood serum and in litmus milk, by the fermentation reaction in glucose media and by the indol reaction in Dunham's solution.

2. Less important differentiating characteristics are found in morphology, motility, colony formation and odor.

Differentiating cultural characteristics

	PEREZ' BACILLUS	B. BRONCHISEPTICUS
Morphology.....	Small coccoid bacillus, no filaments	Small narrow bipolar bacillus. Filaments in liquid media
Motility.....	Sluggish	Active
Colony.....	Old colony, lobate	Round, entire
Loeffler's blood serum....	No proteolysis, cream to greenish yellow	No proteolysis, old cultures tan color
Potato.....	Limited, faintly yellow	Spreading, tan
Plain broth.....	Uniform turbidity, characteristic nauseating odor	Uniform turbidity, odor of stale bread
Litmus milk.....	Small amount of acid	Decided alkalinity
Dunham's.....	Indol positive	Indol negative
Fermentation		
Glucose.....	Acid—gas	Alkaline—no gas

AGGLUTINATION REACTIONS WITH *B. BRONCHISEPTICUS* AND PEREZ' BACILLUS.

Rabbits have been treated with vaccines of the following cultures of *B. bronchisepticus* and Perez' Bacillus and the sera obtained for the purpose of making cross-agglutination tests between these two organisms.

<i>B. bronchisepticus</i> no. 36, from dog	} Isolated by N. S. F.
<i>B. bronchisepticus</i> no. 123, from monkey	
<i>B. bronchisepticus</i> from human	
Perez' Bacillus no. 1.....	Isolated by Ward
Perez' Bacillus no. 2, "Hofer" strain	} European strains
Perez' Bacillus no. 3, "Vienna" strain	
Perez' Bacillus no. 4.....	Isolated by Ward

Vaccines. Each organism was transplanted daily for several days on plain agar; then twenty-four-hour growths were washed off in 0.85 per cent salt solution plus 0.2 per cent trikresol (5 cc. per culture). Each vaccine was thoroughly shaken in a mechanical shaker and two days later tested for sterility.

Production of antisera. Before the rabbits were injected each was bled from an artery in the ear and the serum tested for agglutinins against both *B. bronchisepticus* and Perez' Bacillus.

No rabbit showed an agglutination titre of above 1 in 20 against either organism.

Each rabbit received three intravenous injections of 0.5, 1.0 and 2.0 cc. of killed vaccine three days apart, and was bled to death on the fourth day after the last dose. To the sera was added 0.2 per cent trikresol.

TABLE 1

DILUTIONS	SERUM FROM RABBIT 32, TREATED WITH <i>B. BRONCHISEPTICUS</i> NO. 36 (DOG). AGAINST SUSPENSIONS OF:						
	<i>B. bronchisepticus</i>			Perez' Bacillus			
	No. 36 (dog)	No. 123 (monkey)	Human	No. 1	No. 2	No. 3	No. 4
1-10	+++	+++	+++	-	-	-	-
1-20	+++	+++	+++	-	-	-	-
1-40	+++	+++	+++	-	-	-	-
1-80	+++	+++	+++	-	-	-	-
1-200	+++	+++	+++	-	-	-	-
1-400	+++	+++	+++	-	-	-	-
1-800	+++	+++	+++	-	-	-	-
1-1600	+++	+++	+++	-	-	-	-
1-2000	+++	+++	+++	-	-	-	-
1-3200	+++	+++	+++	-	-	-	-
1-6400	+	+	+	-	-	-	-
1-10000	-	-	-	-	-	-	-
1-20000	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-

+++ = Complete agglutination with all organisms clumped and fluid clear.

++ = Partial agglutination, with marked clumping but fluid not cleared up.

+ = Slight agglutination but still with positive clumping.

- = No clumping and no clearing.

Preparation of suspensions for agglutination tests. Each organism was transplanted daily, on plain agar, for ten days; twenty-four-hour growths planted on plain agar in whiskey flasks; incubated eighteen hours; growths washed off in physiologic salt solution plus 0.5 per cent formalin. The suspensions were shaken for two hours; two days later tested for sterility; then filtered three times through filter paper. Each was later diluted with salt solution plus 0.5 per cent formalin to correspond in density to our standard suspension of *B. bronchisepticus*, which contains about 2,000,000,000 organisms per cubic centimeter. Perfectly homogeneous suspensions of all the strains used were produced by this method.

TABLE 2

DILUTIONS	SERUM FROM RABBIT 36, TREATED WITH PEREZ' BACILLUS NO. 1. AGAINST SUSPENSIONS OF:						
	Perez' Bacillus				<i>B. bronchisepticus</i>		
	No. 1	No. 2	No. 3	No. 4	No. 36 (dog)	No. 123 (monkey)	Human
1-10	++	+++	++	++	+	+	+
1-20	+++	+++	+++	+++	-	-	-
1-40	+++	+++	+++	+++	-	-	-
1-80	+++	+++	+++	+++	-	-	-
1-200	+++	+++	+++	+++	-	-	-
1-400	+++	+++	+++	+++	-	-	-
1-800	+++	+++	+++	+++	-	-	-
1-1600	+++	+++	+++	+++	-	-	-
1-2000	+++	+++	+++	+++	-	-	-
1-3200	+++	++	+++	+++	-	-	-
1-6400	+	+	-	++	-	-	-
1-10000	-	-	-	-	-	-	-
1-20000	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-

TABLE 3

ANTISERA	CROSS AGGLOUTINATION TESTS WITH <i>B. BRONCHISEPTICUS</i> AND PEREZ' BACILLUS. SUSPENSIONS						
	<i>B. bronchisepticus</i>			Perez' Bacillus			
	No. 36 (dog)	No. 123 (mon- key)	Human	No. 1	No. 2	No. 3	No. 4
<i>B. bronchisepticus</i>							
No. 36 {	Rabbit 32.....	1-6400		-	-	-	-
	Rabbit 2.....	1-10000	1-20000	-	-	-	-
No. 123 {	Rabbit 33.....			-	-	-	-
	Rabbit 34.....			-	-	-	-
	Rabbit 4.....	1-10000	1-10000				
Human {	Rabbit 5.....			-	-	-	-
	Rabbit 6.....	1-10000	1-10000	-	-	-	-
<i>Perez' Bacillus</i>							
No. 1 {	Rabbit 36.....	1-10	1-10	1-6400	1-6400	1-3200	1-6400
	Rabbit 37.....	-	-	1-6400			
No. 2 {	Rabbit 38.....	-	1-10	1-10000	1-6400	1-6400	1-10000
	Rabbit 39.....	-	-		1-3200		
No. 3 {	Rabbit 40.....	-	-	1-10000	1-6400	1-10000	1-10000
	Rabbit 41.....	1-10	-			1-6400	
No. 4 {	Rabbit 42.....	1-10	1-20	1-6400	1-6400	1-6400	1-10000
	Rabbit 43.....	1-20	1-20				1-10000

Agglutination tests. The serum was diluted with physiologic salt solution and each tube contained 0.5 cc. suspension plus 0.5 cc. diluted serum. The tests were incubated at 37° C. and readings made at the end of twenty-four hours.

Tables 1, 2 and 3 show the results of the agglutination experiments.

SUMMARY OF AGGLUTINATION EXPERIMENTS.

1. Sera of high agglutination titre (1-3200 to 1-10000) were produced in rabbits by three intravenous injections of killed unheated vaccines of *B. bronchisepticus* and of Perez' Bacillus.

2. All the strains of Perez' Bacillus used were identical by agglutination test—each anti-serum agglutinating all strains equally well.

3. All strains of *B. bronchisepticus* were identical.

4. There was no cross-agglutination between *B. bronchisepticus* and Perez' Bacillus.

COMPLEMENT FIXATION REACTIONS WITH *B. BRONCHISEPTICUS* AND PEREZ' BACILLUS.

Complement fixation tests were made with the same antisera as used for the agglutination reactions.

Technic. The volume of all the complement fixation tests and all titrations was 4.5 cc. The method in general was that given by Kolmer in "Infection, Immunity and Specific Therapy."

For the hemolytic system, *sheep corpuscles in 5 per cent solution* (the blood was defibrinated with beads, measured, washed five times in salt solution, diluted back to original volume and 5 cc. of this used in 100 cc. salt solution), *rabbitamboceptor*, and *guinea pig complement* in a 1 in 20 dilution (serum from at least two pigs being mixed) were used. The amboceptor was titrated daily with each complement and corpuscle suspension, and that amount which showed just complete hemolysis in one hour at 37° C. was taken as the unit. In the tests one and a half times the unit was used.

Each antigen was titrated for its anticomplementary and its antigenic unit. In the tests, one-half to one-fourth the anticomplementary unit was used.

Each antiserum was tested to determine the smallest amount which, with its homologous antigen, completely inhibited hemolysis.

The test. Antigen plus serum plus complement was incubated

TABLE 4

AMOUNT OF SERUM	SERUM FROM RABBIT 32, TREATED WITH <i>B. BRONCHISEPTICUS</i> NO. 36 (DOG). AGAINST ANTIGENS OF:						
	<i>B. bronchisepticus</i>			Perez' Bacillus			
	No. 36 (dog)	No. 123 (monkey)	Human	No. 1	No. 2	No. 3	No. 4
cc.							
0.1	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.	C. H.
0.05	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.	C. H.
0.01	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.	C. H.
0.008	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.	C. H.
0.005	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.	C. H.
0.004	C. I.	Marked I	Marked I	C. H.	C. H.	C. H.	C. H.
0.003	C. I.	Marked I	Marked I	C. H.	C. H.	C. H.	C. H.
0.002	C. I.	Slight I	Slight I	C. H.	C. H.	C. H.	C. H.
0.001	Slight I.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.
Controls:							
Antigen.....	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.
Serum.....	C. H.						
Hemolytic.....	C. H.						
Corpuscle.....	No. H.						

C. H. = Complete hemolysis.

C. I. = Complete inhibition of hemolysis.

one hour at 37° C.; then amboceptor and cells were added and incubated from one to one and one-half hours, depending on the antigen controls. The tests were then placed in the ice-chest and read the following morning.

Antigens. *B. bronchisepticus* and Perez' Bacillus were transplanted daily for several days, then planted on plain agar in whiskey flasks; incubated for twenty-four hours at 37°C.; the growths washed off in sterile distilled water (about 50 cc. per flask); shaken in a mechanical shaker for forty-eight hours; brought up in a water bath to 56° C. and incubated at that

temperature over night: the antigens were then filtered through asbestos and to nine parts of clear filtrate one part of 8.5 per cent salt solution plus 5 per cent formalin was added. The antigens were then kept in the ice-chest.

Cross titrations. Cross titrations were made between the several strains of *B. bronchisepticus*, the several strains of Perez' Bacillus and between *B. bronchisepticus* and Perez' Bacillus.

Each serum was tested against each of the seven antigens and on the same day, all the tests being run parallel.

TABLE 5

AMOUNT OF SERUM	SERUM FROM RABBIT 36, TREATED WITH PEREZ' BACILLUS NO. 1, AGAINST ANTIGENS OF:						
	Perez' Bacillus				<i>B. bronchisepticus</i>		
	No. 1	No. 2	No. 3	No. 4	No. 36 (dog)	No. 123 (monkey)	Human
cc.							
0.01	C. I.	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.
0.05	C. I.	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.
0.01	C. I.	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.
0.008	C. I.	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.
0.005	C. I.	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.
0.004	C. I.	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.
0.003	C. I.	C. I.	C. I.	C. I.	C. H.	C. H.	C. H.
0.002	Marked I.	Marked I.	Marked I.	Marked I.	C. H.	C. H.	C. H.
0.001	Slight I.	C. H.	Slight I.	C. H.	C. H.	C. H.	C. H.
Controls:							
Antigen.....	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.
Serum.....	C. H.						
Hemolytic.....	C. H.						
Corpuscle.....	No H.						

Tables 4, 5 and 6 show the results of the complement fixation tests.

SUMMARY OF COMPLEMENT FIXATION TESTS.

1. Sera of high complement-fixing titre were produced in rabbits by three intravenous injections of killed unheated suspensions of *B. bronchisepticus* and Perez' Bacillus.

2. The four strains of Perez' Bacillus gave identical complement fixation reactions.

3. The human and monkey strains of *B. bronchisepticus* gave identical complement fixation reactions, but these two strains cross-fixed with the dog strain only with larger amounts of serum.

4. Immune sera of *B. bronchisepticus* did not cross-fix with antigens of Perez' Bacillus.

5. Immune sera of Perez' Bacillus did not cross-fix with antigens of *B. bronchisepticus*.

TABLE 6

Cross complement fixation tests with B. bronchisepticus and Perez' Bacillus

ANTISERA	ANTIGENS						
	<i>B. bronchisepticus</i>			Perez' Bacillus			
	No. 36 (dog)	No. 123 (mon- key)	Human	No. 1	No. 2	No. 3	No. 4
	cc.	cc.	cc.	cc.	cc.	cc.	cc.
<i>B. bronchisepticus</i>							
No. 36 {	Rabbit 32.	0.002	0.005	0.005	—	—	—
	Rabbit 2.	0.004	0.008	0.008	—	—	—
No. 123 {	Rabbit 33.	0.008	0.003	0.003	—	—	—
	Rabbit 34.	0.05	0.008	0.008	—	—	—
<i>Human</i>							
Human {	Rabbit 5.	0.05	0.003	0.003	—	—	—
	Rabbit 6.	0.05	0.005	0.005	—	—	—
<i>Perez' Bacillus</i>							
No. 1, Rabbit 36.				0.003	0.003	0.003	0.003
No. 2, Rabbit 38.				0.003	0.003	0.003	0.003
No. 3, Rabbit 40.				0.003	0.003	0.003	0.003
No. 4, Rabbit 42.				0.002	0.002	0.002	0.002

CONCLUSIONS.

According to the cultural reactions and the agglutination and complement fixation tests there appear to be no reasons why Perez' Bacillus should be confused with *B. bronchisepticus*. The organisms can be differentiated from each other by any one or more of the above methods.

The results of Horn and Victors with the complement fixation test have not been corroborated by us.

REFERENCES.

- FERRY, N. S., 1910. A preliminary report of the bacterial findings in canine distemper. *Am. Vet. Rev.*, 37, 499.
- FERRY, N. S., 1911. Etiology of canine distemper. *J. Infect. Dis.*, 4, 399.
- HOFER, G., AND KOFLER, K., 1914. Weitere Mitteilungen über die Ergebnisse der Vakzinationstherapie bei genuiner Ozäna mit einer aus dem *Cocco-bacillus fœtidus ozanæ* Perez. *Arch. f. Laryngol.*, 29, 1.
- HORN, H., AND VICTORS, E. A., 1916. A contribution to the bacteriology of the so-called *Coccobacillus fœtidus ozænæ* (Perez)—, with additional notes on the treatment of clinical ozena by means of polyvalent vaccines made from the same organisms. *Ann. Otol., Rhinol. Laryngol.*, 25, 251.
- M'GOWAN, J. P., 1911. Some observations on a laboratory epidemic, principally among dogs and cats, in which the animals affected presented the symptoms of the disease called "distemper." *J. Path. & Bacteriol.*, 15, 372.
- PEREZ, 1913. Die Ozana eine infectiöse und contagiöse Krankheit. *Berl. klin. Wchnschr.*, 52, 2411.
- TORREY, J. C., AND RAHE, A. H., 1913. Studies in canine distemper. *J. Med. Research*, 27, 291.
- WARD, H. C., 1917. *Coccobacillus (fœtidus) ozænæ* of Perez. *J. Infect. Dis.*, 21, 338.

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AN EXPERIMENTAL STUDY OF SERUM THERAPY IN TRICHINOSIS.

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Salzer¹ has published a study of trichinosis in which he claims to have secured beneficial results in man and in experiment animals by the use of serum from patients recovered from the disease. In this connection he also claims that injections of serum are prophylactic against trichinosis; that animals fed with infected meat within twenty-four hours after the administration of the serum might develop a mild form of trichinosis, but if fed at a later period would prove to be immune; and that if immune serum was mixed with infected meat and then fed to animals, the animals did not develop trichinosis, though the ingestion of the same meat without serum was invariably followed by the appearance of the disease. In two patients in the active stages of trichinosis the administration of the serum showed remarkable curative power; there was a decided drop in temperature within six hours and the abnormal temperature had entirely subsided within twenty-four hours.

Schwartz² has categorically taken up Salzer's claims and rejected them, largely on the basis of animal experimentation, in the following language:

1. Serum from animals convalescent from trichinosis when injected into other animals did not produce immunity to trichinosis in the latter.

¹Salzer, B. F.: *Jour. Am. Med. Assn.*, 1916, 67, 579.

²Schwartz, Benjamin: *Jour. Am. Med. Assn.*, 1917, 69, 884.

2. Trichinous meat mixed with serum from animals during the active or convalescent stage of the disease proved to be still capable of producing the disease. 3. Animals once infected and harboring trichinae in their muscles were not immune to further infection when fed trichinous meat. 4. Serum from a trichinous animal had no observable ill effects on the larvae freed from their cysts by artificial digestion. 5. None of the results of the experiments appear to be in harmony with the assertions made by Salzer concerning the value of serum from convalescent animals as a prophylactic or curative agent in trichinosis.

We have carried out a number of experiments in testing the prophylactic and curative value of serum from animals recovered from trichinosis, and have come to the following conclusions: So far as our experiments are comparable to those performed by Schwartz, our findings are in agreement with his and tend to disprove Salzer's specific contentions along these lines, but we believe that, in spite of some erroneous contentions, Salzer may be right in part of his assertions and the treatment he suggests worthy of more approval than Schwartz is inclined to accord to it.

The reason we both agree and disagree with Salzer and with Schwartz is that there are two phases of trichinosis which are not differentiated by either of these writers, though each stands on good ground according to the phase he is considering. Trichinosis is a disease due to the presence in the body of the worm called *Trichinella spiralis* (*Trichina spiralis*). One phase of the disease is a mechanical phase, due to the presence and actions of the worm in the digestive tract, the blood stream, and the musculature. The other phase of the disease is chemical in nature and is due to the presence in the blood of the more or less toxic excretions and secretions of the worm. The first phase of the disease sets up an inflammatory defense reaction which terminates in the walling-in and sealing of the worm cyst in the muscles. The second phase of the disease sets up the customary antibody defense reaction of the blood, which defense reaction becomes manifest in an eosinophilia that may go as high as 86 per cent and which presumably terminates when the sealing of the worm cyst prevents the excretion and secretion products from reaching the blood stream and when those already present are neutralized or otherwise disposed of and rendered innocuous by the blood.

As regards the first phase, in our opinion, serum has little or no power to prevent the development of the larval worms to adults in the intestines, to prevent the embryo worms from invading the blood stream and tissues, and to prevent these embryo worms from encysting and developing to infective larvae. The experiments performed by Schwartz and ourselves bear out this contention, and so far as Salzer claims the contrary we believe he is in error.

The claim is occasionally made that worm infestations produce immunity to subsequent infestations with the same worm. For most worms there is a great deal of evidence against the belief and but a small amount in favor of it. It has recently been claimed by Fujinami for schistosomiasis.

As regards the second phase, there seems to be no evident reason why serum from an animal that has recovered from trichinosis should not be of value in protecting another animal from the toxic effects of the secretions and excretions of the worms. These materials, be they specific secretions or waste products of the worm's metabolism, must act like any other foreign protein in the blood in the production of antibodies, and recovery from the disease represents not only the survival of the mechanical damage due to the worm, but an adequate production of antibodies to offset the deleterious effect of these toxic proteins on the body economy. In this connection it might also be noted that while worm infestations in general are typically afebrile conditions, trichinosis in man is typically febrile, and that the production of the high temperatures has been attributed by some workers to the invasion of the tissues and blood stream by bacteria accompanying the worms. There is exactly the same reason to suppose that the serum of an animal which has recovered from trichinosis would be of value in supplying readymade antibodies to combat toxic worm products, and perhaps accompanying bacterial products, in a case of trichinosis, as there is for believing that serums from immune animals are of value in diphtheria or tetanus. In this respect we are in agreement with Salzer as to the probable value of such a serum, and believe that Schwartz has not attached enough importance to this phase of the subject.

EXPERIMENTS.

In undertaking our experiments we first infected a dog in order to obtain a supply of serum. The trichinous pork for feeding to start the experiments was obtained through the kindness of Dr. B. H. Ransom, chief of the Zoological Division of the United States Bureau of Animal Industry, who has made some notable contributions along the line of control of trichinosis through the federal meat inspection service. The infected meat was fed in small lots to two dogs, as follows:

Doc. 127—A mongrel hound weighing 11.5 kg. was given 2 gm. of the meat. Daily examinations of the feces for the next twenty-six days did not show the passage of any trichinae. On the twenty-sixth day the dog was bled from the jugular and then killed with chloroform. Examination of the diaphragm did not disclose any trichinae. The animal may have had a light infection, which was not detected, or it may have been immune to trichinosis, as dogs are commonly resistant to the development of trichinae. Such resistance to infection with species of worms other than those customarily found in a given host species seems to be more a matter of nice adaptation to host morphology, temperatures, chemical reaction of the gastro-intestinal secretions, and such inherent physical and chemical conditions, than a matter directly related to any of the blood reactions commonly concerned in immunity.

Doc. 129—A mongrel weighing 15 kg. was given 3 gm. of trichinous meat; twenty-five days later it was fed 2 gm. more; ten days later it was fed 2 gm. more; eleven days later it was fed 4 gm. more; four days later it was fed 4 gm. more; five days later it was fed 5 gm. more; six days later it was fed 5 gm. more; eight days later it was fed 64 gm. more, a total of 89 gm. of trichinous meat. The dog remained active throughout the experiment and did not appear diseased or uncomfortable except for a keratitis beginning the second day after the first feeding and disappearing eighteen days later. The dog was bled from the jugular under chlorotene anesthesia 111 days after the first feeding and forty-two days after the last feeding, and the serum separated and preserved with 0.4 per cent trikresol. The diaphragm was found to be fairly well infested with trichinae.

The animals used for the tests of the serum were white rats. They are easy to infect, but they show little clinical evidence of the disease, so that our tests of efficacy are primarily a consideration of the longevity of infected rats treated with serum in comparison with check animals similarly infected but not treated, all animals being kept, so far as possible, under identical conditions

of temperature, food, water, etc. The rats were run in series of two to six, one or more serving as checks, and the others receiving various treatments.

We tried guinea-pigs first, feeding them from the same trichinous pork with which the dogs had been fed, but experiments with twelve of them showed that the meat was but slightly infective at this time or the guinea-pigs were resistant, so the diaphragm of Dog 129 was used as the initial infective material and the guinea-pigs dropped in favor of rats. The supply of trichinous meat was kept up by feeding dead experiment rats to new experiment rats. The experiments follow:

Series 1.—To Determine Whether Serum Affects Trichina Larvae.

Rat 1 was fed 10 gm. of the diaphragm of Dog 129 and six days later was fed 13 gm. more. Thirty-four days later the rat received a subcutaneous injection of 1 c.c. of the serum from Dog 129. The rat was killed nine days later and the muscles found infested. It was used to infest other rats.

Rat 2 was fed the same amounts at the same time as Rat 1. It was injected the same day, but received 2 c.c. of serum. It died forty-four days after the injection, and the muscles were found infested and infective for other rats.

This experiment only indicates that serum exerts no evident bad effect on trichinae when used at the interval and in the amount given, the trichinae being alive and infective for susceptible animals.

Series 2.—To Determine the Effect of Simultaneous Infection and Feeding.

Rat 1 was fed 1 gm. of meat from Rat 1 of Series I, and was injected at the same time with 1 c.c. of serum. The rat escaped while a temperature was being taken and was soaked with cold water before it was captured. It was found dead the next day. Postmortem examination showed pneumonia. This animal cannot be considered in determining the results of the experiments.

Rat 2 was fed 1 gm. of meat from Rat 1 of Series 1, and was injected at about the same time with 2 c.c. of serum. The following day it was fed 1 gm. more of the meat. Two days after this second feeding the rat was almost dead, so it was killed. The intestine was hemorrhagic and scrapings showed numerous trichinae.

Rat 3, the check, was fed the same amounts and at the same time as Rat 2, but was given no serum. This rat died either the afternoon of the day it was fed the second time or the following day. The intestine was hemorrhagic and showed numerous trichinae, especially in the duodenum.

In this experiment the treated animal outlived the check a short time, one or two days.

Series 3—To Test the Prophylactic Value of Serum Simultaneously Injected or on Food.

Rat 1 was fed 2 gm. of trichinous meat and was injected at the same time with 1.5 c.c. of serum. Eight days later it was fed 1 gm. more. Forty days after the second feeding it was fed 1 gm. more. Six days after this last feeding the rat was killed. The muscles were heavily infested.

Rat 2 was fed the same amounts and at the same time as Rat 1, but the first feed of trichinous meat was mixed with 5 c.c. of serum before feeding. In addition to the feedings at the same time as Rat 1, this rat was fed 1 gm. of meat the day Rat 1 was killed. Four days later it was fed 1 gm. more; six days after this feeding it was fed 1 gm. more. Five days after the last feeding it was found dead. The intestine was hemorrhagic and contained trichinae. Trichinae were abundant in the muscles.

Rat 3, a check, was fed the first two times that the others were fed, getting the same amount of trichinous meat. Two days after the second feeding the rat was found dead. No trichinae were found in the diaphragm or intestines.

In this experiment the rats receiving the serum outlived the check rat decidedly, but it was impossible to associate definitely the death of the check animal with the feeding of trichina. Experiments show, however, that neither the injection nor the feeding of serum prevented infection with trichinae.

Series 4—To Test the Prophylactic Value of Serum Simultaneously Injected or on Food.

Rat 1 was fed 1 gm. of meat mixed with 1 c.c. of serum. Additional feedings of 1 gm. each were made on the third, tenth, fourteenth and twentieth days after the first feeding, but no serum was given with these feedings. The day after the last feeding the rat was found dead. The intestines were hemorrhagic and the lungs pneumonic. Trichinae were found in the intestines and musculature.

Rat 2 was fed the same amounts and at the same time as Rat 1, but the first gram of meat was mixed with 2 c.c. of serum. Following the death of the first rat, No. 2 was fed additional meat as follows: 24 days after the first feeding, 0.5 gm.; 28 days, 1 gm.; 38 days, 1 gm.; 45 days, 2 gm.; 52 days, 1 gm.; 57 days, 1 gm.; 63 days, 1 gm.; 68 days, 0.5 gm. The rat died the day after the last feeding. The lung was pneumonic and presented tubercles, the nature of which was not determined. There were trichinae in the intestine and the musculature showed a very heavy

infestation. Trichinae were present in the scalp muscles, the eye muscles and in some coats of the eye.

Rat 3 was fed the same amounts and at the same time as Rat 1, but received an injection of 1 c.c. of serum when the first gram of meat was fed. This rat died five days after Rat 1. The stomach and small intestine were hemorrhagic and the lungs pneumonic. There were trichinae in the intestine and musculature.

Rat 4 was fed the same amounts and at the same time as Rat 2, but received an injection of 2 c.c. of serum at the time the first gram of meat was fed. This rat also received an additional 0.5 gm. of meat seven days after the last feeding of Rat 2. Ten days after this feeding the rat was found dead. This rat showed a pronounced edema of the eyelids and the left eye became purulent and blind. Trichinae were found in the intestine and the muscles were heavily infested.

Rat 5, a check, was fed the same amounts and at the same time as Rat 1, but received no serum. In addition it was fed 0.5 gm. of meat four days after the last feeding of Rat 1, and 1 gm. eight days after the last feeding of Rat 1. Two days later the rat began to develop nervous symptoms. It would fall to the right side and could hardly assume or maintain an erect position. Four days later the condition was more marked and the rat would roll over and over to the right unless restrained by some limiting object on that side. Eight days after its last feeding the rat was found dead. The intestine was hemorrhagic and the lungs pneumonic. Trichinae were found in the intestine and in the diaphragm.

Rat 6, a check, was fed the same amounts and at the same time as Rat 5. In addition it was fed 1 gm. of meat ten days after the last feeding of Rat 5. Eight days after its last feeding the rat was found dead. There was a severe hemorrhagic condition in the jejunum, less severe in the duodenum, and the lungs were pneumonic. Trichinae were found in the intestine and diaphragm.

It will be seen from the foregoing that the serum injected or fed with the trichinous meat had no evident prophylactic value.

As regards the curative value of the serum, as judged by comparative longevity of the treated animals and the checks, we find the following:

Two of the treated animals, those receiving the largest amounts of serum by injection or feeding, outlived both the checks, and both the checks outlived the other two treated animals, those receiving the smallest amount of serum by injection or feeding. As the feedings were kept up, the survivors in all cases received larger amounts of meat than the animals outlived by them. The animals receiving the larger amount of serum outlived the checks by totals of 120 days, while the checks outlived the animals receiving the smaller amount of serum by totals of eighty-four days. This leaves a slight presumption in favor of the idea that there is some value in the treatment. The severity of infestation in Rats 2 and 4 may be fairly associated with their additional feedings.

Series 5.—To Test the Prophylactic Value of Simultaneous and 3 and 4 c.c. Injections of Serum.

Rat 1 was fed 2 gm. of meat and at the same time was given a subcutaneous injection of 3 c.c. of serum. Seven days later it was fed 2 gm. more of meat; seventeen days later, 2 gm. more; twenty-four days later, 1 gm. more, and twenty nine days after the first feeding was fed 1 gm. more. Two days later the rat was found dead. The lungs showed hepaticized areas, the liver was light colored and apparently degenerated. Trichinae were found in the intestine, and the diaphragm was rather lightly infested.

Rat 2 was given two feedings at the same time that Rat 1 received its first two, and got the same amounts, but was given an injection of 4 c.c. of serum at the time of the first feeding. Seventeen days after the first feeding it was found dead. The lungs showed areas of hepaticization and there was some blood in the intestine. Trichinae were plentiful in the intestine, but the diaphragm was very lightly infested.

Rat 3, a check, was fed four times at the time Rat 1 received its first four feedings, and received the same amounts as Rat 1. Five days after the last feeding this rat was found dead. The lungs were pneumonic and the jejunum hemorrhagic. There were trichinae in the intestine and the diaphragm was very heavily infested.

While the injections of large amounts of serum, 3 or 4 c.c., did not prevent infestation with trichinae in Rats 1 and 2, it is nevertheless interesting to note that these rats, which had been fed five times and two times, had very light infestation with trichinae, while the check rat, which was fed four times, had a very heavy infestation.

As regards longevity, a treated rat outlived the check nine days and the check outlived the other treated rat five days, a margin in favor of the treated animals.

Series 6.—To Test the Prophylactic Value of Injections Previous to Feeding.

Rat 1 was injected with 1 c.c. of serum, and fed 1 gm. of heavily infested meat the next day. One week after each of these proceedings, the injection and the feeding were repeated. Five days after the second injection of serum it was repeated, followed by the feeding of a gram of the meat the next day, making a total of three injections of 1 c.c. of serum followed each time by feeding 1 gm. of meat the next day. Four days after the last feeding the rat was found dead. The lungs were pneumonic. A moderate number of trichinae were found in the intestine, but none in the diaphragm at that date.

Rat 2 received the same treatment, as regards serum and trichinous meat, that Rat 1 received. This animal lived and enjoyed good health for fifty-eight days and was killed at that time to permit of postmortem examinations and to close up the experiment. The anterior portion of the right lung presented tubercles and was adherent to the chest wall at

this point. There were no trichinae found in the intestine and only a very moderate number in the diaphragm.

Rat 3, a check, was fed the same amount of trichinous meat at the same time as the other rats, but was given no serum. This rat died five days after the last feeding. The intestine was hemorrhagic and heavily infested with trichinae. No trichinae were found in the diaphragm at this time.

As regards longevity, the check outlived one treated animal by one day, but the other treated animal outlived the check by thirty-nine days and was killed when it had evidently outlived any danger from its trichina feeding and apparently would have lived much longer.

As regards development of trichinae, the check rat had a much larger number in the intestine than the treated Rat 1; these two rats did not show somatic trichinosis, but died too soon to permit of conclusions in regard to this. Rat 3 lived long enough to lose its intestinal trichinosis and had acquired a mild somatic infestation.

SUMMARY OF EXPERIMENTAL DATA.

A summary of the foregoing data on longevity shows: Of fifteen rats, those given the serum treatment outlived the checks in nine cases, whereas the checks outlived those given the serum in six cases. In two cases the treated animals were killed instead of being allowed to die. The figures for each series are as follows:

Series 2: Treated animal outlived the check by one or two days.

Series 3: Treated animals lived an average of 62.5 days; the check, 11 days. Treated animals lived on an average 51.5 days longer than the check.

Series 4: Treated animals lived an average of 51.25 days; checks, 45.5 days. Treated animals lived on an average 5.75 days longer than the checks.

Series 5: Treated animals lived an average of 25 days; the check, 23 days. Treated animals lived on an average 2 days longer than the check.

Series 6: Treated animals lived on an average 37 (plus) days; the check, 18 days. Treated animals lived on an average 19 (plus) days longer than the check.

Note the treated animals outlived the checks in every series, the total number of days which treated animals survived the checks being 80 (plus).

From what we know of antibody production it appears, then, entirely likely that serum from an animal recovered from trichinosis would be of value in combating the toxic features of the disease. Our experiments seem to bear out this idea. The experiments are by no means proof of the value of the treatment, but they are evidence which accords with the theoretic possibilities and with Salzer's good clinical results in two of his cases, which results deserve to be considered apart from what appear to be undemonstrable claims in regard to serum inhibition of *trichina* development.

The clinical picture of trichinosis in rats is not a well defined affair as in man. Rats are the habitual and normal hosts of trichinae and probably have an inherent tolerance for these worms. Raebiger³ found no eosinophilia in the blood of trichinous rats and but few eosinophils around the encysted trichinae, though Opie⁴ found eosinophilia beginning at the end of the second week. Rats show little evidence of myalgia, and the pyrexia present in man seems to be lacking. The day after rats are fed trichinae there is usually a marked fall of temperature, followed by a rise to normal the following day, or a slow rise for several days. Such data as we have on this point (temperatures were not taken on Sundays) show that twenty-four hours after feeding trichinous meat without serum in any form, the temperature dropped 42 times, an average drop of 1.9 degrees, and rose 19 times, an average rise of 1.1 degrees. Where trichinous meat was fed and serum injected at the same time the temperature dropped 5 times, an average of 0.96 degree, and rose once, 1 degree. Where trichinous meat was fed after being mixed with serum the temperature dropped in both of two cases, 0.3 and 1.2 degrees. Where serum was injected without trichinous meat being fed the same day the temperature dropped 4 times, an average of 0.56 degree, and rose 4 times, an average of 0.5 degree. The greatest fall of temperature followed by recovery was in the case of Rat 1 of Series 4; on the third feeding the temperature dropped from 99.8 F. the day of feeding to 95.5 the following day, a drop of 4.3 degrees, but rose to 99 the next day. The greatest rise in temperature was in the case of Rat 4 of Series 4;

³ Raebiger, S. *Trichinosis in the Rat, *Rattus norvegicus**. 1911, p. 120.
⁴ Opie, L. L. *Ann. Surg.* 1908, 47: 477.

on the sixth feeding the temperature rose from 97.6 to 101, a rise of 3.4 degrees.

The usual fall in temperature seems to be associated with a marked enteritis. There is usually blood in the feces and rectum the day after feeding trichinous meat, a condition that may visibly persist for as many as four days after feeding. Associated with this is a diarrhea and more or less gas in the intestine. Pneumonic conditions were rather common in these rats, perhaps due to the passage of embryos through the pulmonary capillaries. The edema of the eyelids, which is comparatively common in trichinosis in man, was only detected once in our rats.

The fact that rats are extremely tolerant of trichinae and do not present a clinical picture comparable to that in man, and the fact that other experiment animals are open to the same objection (experimental feeding to a shoat failed to develop pyrexia, though 83 gm. of trichinous meat were fed in thirty-three days) or are open to the objection that they are difficult to infect, suggests that the value of immune serum in trichinosis can hardly be ascertained except by clinical test on human patients. The lack of febrile conditions in rats, or in the shoat just mentioned, suggests that in these animals there is an inherent immunity to the second phase of trichinosis, the phase associated with toxic products in the blood and with antibody production against the toxins. Rats and swine have presumably acquired this immunity through ages of infestation and apparently suffer primarily from the first phase of the disease, the mechanical phase, against which no immunity could readily be developed. Man, in a comparatively short and limited experience in feeding on raw trichinous pork, has developed no such immunity to the second phase of trichinosis.

In view of the fact that our present day treatment of trichinosis is mostly palliative and symptomatic, a serum treatment might be of considerable therapeutic value. Its commercial value would be slight. The number of cases of trichinosis in the United States yearly is not large; the serum from one horse which had been properly immunized by feedings of trichinae would probably more than supply treatment for all cases. Such production could only be profitably undertaken by a firm selling directly from a central plant; it could not be profitably under-

taken by a firm with numerous branch houses to be restocked yearly.

SUMMARY AND CONCLUSIONS.

Our experiments bear out the conclusions of Schwartz to the effect that serum from animals convalescent from trichinosis, when injected into other animals or fed to them with trichinous meat, does not inhibit the customary development of trichinae.

On the other hand, theoretic considerations, the clinical observations of Salzer, and the longevity data from our experiments lead us to the conclusion that such a serum may be of decided value in combating the toxic features of trichinosis, a conclusion which is in general agreement with Salzer's belief in the value of such a serum.

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**THE COMPARATIVE VALUES OF SOME LOCAL
ANESTHETICS.**

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Novocaine, while conceded to be somewhat less effective in practice than cocaine, has gained a reputation for effectiveness which, taken along with its stability in solution when heated to sterilize, its lower toxicity, and its freedom from habit-forming effects, has made it second to none in its general applicability. While it is true that most of the defects of cocaine can be overcome by one means or another the disappearance of novocaine from the market has been generally regarded as a distinct loss.

A recent addition to the list of local anesthetics is apothesine, which has proved to be not merely a substitute for novocaine and cocaine but a product worthy of closest investigation. Unfortunately, in a series of experiments by Sollmann¹ in which he examined some of the most important local anesthetics this product was passed over as of minor importance.

The reason advanced by Sollmann for reopening an investigation of local anesthetics, namely "the wish to select 'the best,'" is sufficient reason for this publication, with the added incentive that while his work is apparently comprehensive, covering as it does the efficiencies of a number of substances by five different methods of valuation, the whole field is not fully covered, since apothesine is included in the tests of only two of the five series.

The first series of his experiments, and the first one appearing in complete detail,² is that describing the comparison of a number of local anesthetics on the sciatic nerve of the frog. One would assume from reading the first article that apothesine had not come to the author's attention.

In two subsequent test-methods, however, it is included—in the cornea test and in the intracutaneous test. Based on the results obtained by the first of these two methods he concluded that novocaine and apothesine are inefficient relatively to cocaine, although apothesine is 100 per cent more active than novocaine. In the intracutaneous test his results would indicate that novocaine is fully equal to cocaine while apothesine is only one-eighth as efficient.

Since Sollmann's experiments led him to conclusions radically different from those which we have observed, the subject is reopened to include the comparison of apothesine with other anesthetics by three of the methods he applied and to point out what are evidently errors in his work.

As in Sollmann's paper, this publication does not touch on the toxicity, since there are different interpretations to be placed on results obtainable by almost any method. The important feature in this respect is that apothesine is only one-fifth as toxic as cocaine by subcutaneous injection into guinea pigs and that in relatively large doses no systemic effects have been in evidence. Local irritation, too, is a clinical problem and may logically be omitted from this discussion.

As to the relative merits of the different methods of examination of local anesthetics, this too must be left to the ultimate judgment of the clinician. The use of a new anesthetic is necessarily governed by its relative efficiency, first of all as shown by laboratory tests but finally by observation of its action in practical use.

Among the laboratory tests that have been devised for investigation of local anesthetics there are three, each of which appears to have a very direct bearing on the clinical application of these substances, namely: the intracutaneous, comparable to infiltration anesthesia; the nerve trunk anesthesia, comparable to nerve blocking or conduction anesthesia; and surface anesthesia—the mucous membrane of the eye, nose and throat—reproduced by application to the frog's foot or to the cornea.

If the results of testing the different local anesthetics by the different methods agreed relatively, this would show that comparison by any one of the methods would give the relative

efficiencies. That this is not true, however, is apparently shown by observing the varying ratios obtained by both Sollmann and ourselves. The laboratory results are therefore only suggestive, and do not absolutely demonstrate the clinical value of a local anesthetic.

In the following experiments the hydrochlorides of each of the local anesthetics tested were used in every case, no experiments having been conducted after neutralization. Experiments with mixed anesthetics were also omitted as having no direct bearing on the object of the paper.

MOTOR NERVE ANESTHESIA.

The method most commonly used for testing the value of a local anesthetic where exact data are desirable, is to apply the anesthetic to the sciatic nerve of the frog, the nerve being separated at its point of branching from the spinal column. The objection has been made that this is a determination of motor nerve anesthesia only and no evidence of efficiency on the sensory nerves. It has been demonstrated, however, that the sensory nerves are the first to be anesthetized³ and therefore a measure of motor nerve anesthesia is a fair indication that the sensory nerves are at least equally anesthetized. Objection has also been made that this method is very different from the practical use of anesthetics, but on this point, too, there are counterbalancing reasons which give the method practical value for measuring anesthetics. This test shows at once the nerve blocking value such as results from subcutaneous and subdural injections, and as this is one of the uses of local anesthesia its measurement is of primary importance. This method also meets one of the requirements of physiological testing in its accuracy of measurement, the exposed nerve can so easily be irritated to determine the degree of anesthesia when either the strength of solution or the length of time is the measuring factor. The accuracy of measurement is not affected by the factors which may affect the intracutaneous method, one of which is the pressure of the solution and another its character, such as tonicity, concentration, irritation, diffusibility.

As a sole method of valuing local anesthetics it would fail to give a true picture, but taken as one of several methods, especially along with that of subcutaneous administration, the nerve blocking action is undoubtedly of primary importance and deserves especial consideration.

This phase of the subject has been so thoroughly covered by other investigators that no further description is necessary and only additional data or materially different results furnish an adequate reason for further publications.

While working with the intent to verify or disprove Sollmann's results the method followed was varied to the extent that no work was carried out with the high dilutions he employed, these having no practical bearing on the subject.

TABLE I.

ANESTHESIA AS SHOWN BY RESPONSE TO CURRENT AFTER MINUTES.

SUBSTANCE	SOLUTION %	0	5	8	10	12	15	20	25
Cocaine	0.5	-	-	±	±	+			
		-	-	±	±	±	±	±	+
		-	-	±	±	±	±	+	
Cocaine	1.0	-	-	±	+	+			
		-	-	±	+	+			
		-	-	±	±	+			
Novocaine	0.5	-	-	±	±	±	±	+	
		-	-	-	±	±	±	±	+
		-	-	±	±	±	±	+	
Novocaine	1.0	-	-	±	±	+			
		-	-	±	-	+			
		-	±	±	±	+			
		-	-	±	±	+			
		-	-	±	±	+			
Apothesine	0.5	-	-	±	±	±	+		
		-	±	+	±	±			
		-	-	+	±	±	±	+	
		-	-	+	±	±	±	±	+
		-	-	±	±	±	±	±	
Apothesine	1.0	-	-	±	-				
		-	-	±	-				
		-	±	±	-				
		-	-	±	±				
		-	-	±	+	+			
KCl	0.5	-	-	-	-	±	+	±	+
	1.0	-	±	±	+	+			

TABLE II.

TIME IN MINUTES REQUIRED FOR COMPLETE ANESTHESIA OF THE WHOLE LENGTH OF THE SCIATIC NERVE.

	0.5% SOLUTION	1% SOLUTION
Cocaine	12-25	10-12
Novocaine	20-25	10-12
Apothesine	15-25	10-12
Potassium Chloride	25	12

It was planned to use such dilutions only as would bring about complete anesthesia in five to ten minutes, but the lower time limit was in no case sufficient with the dilutions used.

The irritation was produced from a three-cell battery through a secondary with 5000 windings adjusted to a point where the current is just perceptible on the tongue.

The sciatic nerve of the frog is dissected, leaving a small piece of the bone attached, for convenience in handling, and having the whole muscle below the knee skinned but otherwise intact.

After determining that its response to stimulation is good, the whole nerve-muscle preparation is immersed in the solution, testing frequently after five minutes to determine when anesthesia begins and its progress. It will be observed that no account is taken of certain features which have formed an important part of the reports of other investigators, namely, the response to varied degree of current, the response from application of stimulus to different portions of the nerve, the exact beginning of paralysis, its duration or the recovery. While these factors are not unimportant this series of tests was made primarily to compare the efficiency of several local anesthetics, and for this purpose one end point may be considered as good as another, other things being equal. The end point chosen was therefore that dilution which would paralyze the motor nerve all the way to its disappearance into the tissue below the knee, in a period of approximately ten minutes. However, for comparison and as evidence of different degrees of anesthesia, records of other times and of other dilutions are included.

In the table, - means practically no degree of anesthesia, ± means partial anesthesia in the sense of progress along the

nerve. ' means complete anesthesia except the nerve just at its disappearance into the tissue of the leg.

From Table I the efficiencies of the four anesthetics are apparently so nearly equal that for all practical purposes their values by this method may be taken as equal, as shown by Table II which summarizes the results.

Comparison of these results with those of Sollmann shows that there is absolute agreement in ratio so far as each of us carried our experiments but that the efficient concentrations used are somewhat different. This is probably due to the selection of different constants and not to errors on either side.

The added data on apothesine are valuable, since the virtual agreement on the others points to a probable agreement on this substance.

SURFACE ANESTHESIA

The method is that of Gradenwitz⁵ who first applied it as a means of determining the relative values of local anesthetics. The brain, medulla and heart of the frog are removed and the blood washed out to prevent general absorption of the substance which is being tested. In applying this method, one leg, after removing excess moisture, is dipped into the diluted anesthetic for 1 minute, after which it is laid in a vessel containing cold water but with the legs not immersed and separated so that none of the anesthetic touches the control leg. At five-minute intervals both feet are dipped into a weak solution of hydrochloric acid (about 1.5 per cent) and the behavior closely observed. When no anesthesia has taken place both legs are withdrawn with equal promptness, while as anesthesia progresses the treated leg is more slowly withdrawn until on complete anesthesia it is not withdrawn at all while the control leg reacts as promptly as at first. After each stimulation both legs are dipped in water to remove any adhering acid.

The objection has been raised that there is no direct relationship between this method and clinical practice; on the other hand the frog's skin being always moist is in a sense comparable to the mucous membrane and, in this respect, is an experimental method for obtaining relative values for mucous membrane anesthesia. Besides, it is a method with a reaction susceptible of

quite exact measurement and as such takes its place as a valuable pharmacologic assay method for establishing relative values. The values so obtained may not be capable of direct application, as has been stated about other methods, but without question they are not to be ignored in assigning the real position of a local anesthetic.

TABLE III.

		TIME AND RESULT OF OBSERVATION.									
SUBSTANCE	DILUTION	5	10	15	20	25	30	35	40	45	
	‰										
Cocaine	2	+	+	+	+						
	2	+	+	+	+			0			
	1	+	+	++			0				
	1	+	++	++	±	±	±	±	0		
	½	±	±	±	±	+++	0				
	½	±	+++	+++	++	+++	+++	0			
	2/3	—	±	±	++	+++	+++	+++	+++	0	
Novocaine	2	—	±	±							
	2	—	±	±			0				
	2	—	±	±			0				
	3	—	±	++	++	±	±	+	0		
	3	—	—	—	±	±	++	++	±		
	3	—	±	++	+	0					
	3	—	±	++	++	++	0				
Apothesine	2‰	0	±	±	±	+	0				
		—	±	±	—	0					
		±	±	±	++	++	±				
	3	—	±	++	++	++	+	+	+++		
	3	—	±	++	++	++	+	0			
	3	++	±	++	++	++	+	+	0		
	3	—	+++	+++	+++	0					
KCl	1	—	±	±	—	—	0				
	2	—	±	±	±	±	%—	0			
	3	+	+	+	++	++	++	++	0		
	2½	±	++	±	++	++	++	0			

SUMMARY OF TABLE IV.

SUBSTANCE	EFFECTIVE DILUTION	RATIO OF EFFECTIVENESS
Cocaine Hydrochloride	1 per cent	1
Novocaine	3 per cent	⅓
Apothesine	3 per cent	⅓
Potassium Chloride	3 per cent	⅓

Table III shows the detailed results in which equally prompt withdrawal of the legs is represented by —, progressive anes-

thetia by \pm while complete anesthesia of the treated member only is represented by $+$. When neither leg will respond to stimulation this condition is represented by 0.

Comparing these relative efficiencies with those obtained by Sollmann it is seen that he found it necessary to use a solution of novocaine six times as strong as that of cocaine as against one three times as strong in Table III. Also that of KCl, the efficient strength was twelve times as great as for cocaine against three times in Table II.

INTRACUTANEOUS METHOD

This method has the distinct advantage of most nearly duplicating one of the important uses of a local anesthetic, since it is carried out in a way almost identical with that in clinical practice. Particular care was taken in the tests tabulated below to prevent the unintentional prejudice which knowledge of what was injected might have.

The injections were made by a practicing physician into the forearms of the subjects, who were asked to respond only when pain was felt when a needle prick was made on the anesthetized area or the adjacent skin.

EXPERIMENTS ON FIRST SUBJECT (H. C. H.)

First Series.

	AMOUNT	DILUTION	RESULT
Cocaine	0.25 c.c.	1-500	Complete
Novocaine	0.25 c.c.	1-200	Complete
Apothesine	0.25 c.c.	1-200	Complete
Phys. Salt Sol.	0.25 c.c.		No anesthesia

Anesthesia was so complete that no distinction could be drawn between the different injections. The second series was therefore made with the same ratio of strengths but each reduced to half that in the preceding series.

Second Series

	AMOUNT	DILUTION	RESULT
Cocaine	0.2 c.c.	1-1000	Complete
Novocaine	0.2 c.c.	1-400	Complete
Apothesine	0.2 c.c.	1-400	Complete

In this test while anesthesia was complete in every case it seemed more persistent in the area injected with apothesine and in the next series the strength of the latter only was changed.

Third Series.

	AMOUNT	DILUTION	RESULT
Cocaine	0.2 c.c.	1-1000	Complete
Novocaine	0.2 c.c.	1-400	Complete
Apothesine	0.2 c.c.	1-800	Complete

In this series the anesthesia was as nearly identical as impartial observation could recognize.

The experiments were continued the next day on the same subject.

In the data below an attempt is made to indicate the degree of anesthesia and its duration by the number of plus signs.

First Series.

	AMOUNT	DILUTION	RESULT
Cocaine	0.2 c.c.	1-1000	++++
Novocaine	0.2 c.c.	1-500	+++
Apothesine	0.2 c.c.	1-1000	++++

Second Series.

Cocaine	0.2 c.c.	1-2000	++
Apothesine	0.2 c.c.	1-2000	+

Third Series.

Cocaine	0.2 c.c.	1-2000	++
Apothesine	0.2 c.c.	1-1500	++

The above injections were made with not to exceed a minute interval between those of a series.

The results of these tests are summarized in Table V.

TABLE V.

SUBSTANCE	DILUTION	RATIO
Cocaine	1-2000	1
Novocaine	1-1000	$\frac{1}{2}$
Apothesine	1-1500	$\frac{3}{4}$

Experiments on a second subject (M. W.) were carried out, the degree of anesthesia being indicated roughly by the number of plus signs.

EXPERIMENTS ON SECOND SUBJECT.

First Series.

	DILUTION	RESULT
Cocaine	1-2000	+++
Novocaine	1-1000	++
Apothesine	1-2000	+++

Second Series.

Cocaine	1-3000	++
Novocaine	1-2000	+
Apothesine	1-3000	++
Novocaine	1-1000	++

These results may be summarized in Table VI.

TABLE VI (M. W.)

	DILUTION	RATIO
Cocaine	1-3000	1
Novocaine	1-1000	$\frac{1}{2}$
Apothesine	1-3000	1

On a third subject (W. E. K.) the results were as follows:

EXPERIMENTS ON THIRD SUBJECT.

First Series.

	DILUTION	RESULT
Cocaine	1-3000	+++
Novocaine	1-1800	++
Apothesine	1-3000	+++

Second Series.

Cocaine	1-4000	++
Novocaine	1-2000	+
Apothesine	1-4000	++

TABLE VII (W. E. K.)

SUMMARY ON THIRD SUBJECT.

SUBSTANCE	DILUTION	RATIO
Cocaine	1-4000	1
Novocaine	1-1800	less than $\frac{1}{2}$
Apothesine	1-4000	1

EXPERIMENTS ON FOURTH SUBJECT (L. W. R.)

First Series.

	DILUTION	RESULT
Cocaine	1-2000	+++
Novocaine	1-1000	++
Apothesine	1-2000	+++

Second Series.

Cocaine	1-3000	++
Novocaine	1-1800	++
Apothesine	1-3000	+

Third Series

	DILUTION	RESULT
Cocaine	1-4000	+
Novocaine	1-1800	++
Apothesine	1-4000	+

Fourth Series

Cocaine	1-3000	++
Apothesine	1-3000	++

Summarizing these results the ratios are shown in Table VIII.

TABLE VIII (L. W. R.)

SUBSTANCE	DILUTION	RATIO
Cocaine	1-3000	1
Novocaine	1-1500	$\frac{1}{2}$
Apothesine	1-3000	1

TABLE IX.

SUMMARY OF AVERAGE RESULTS BY INTRACUTANEOUS ADMINISTRATION.

SUBSTANCE	RATIO
Cocaine	1
Novocaine	11/24
Apothesine	15/16

The results of the intracutaneous administration of the three local anesthetics summarized above, carried out as they were on four different persons, prove conclusively two points: (1) novocaine is about one-half as efficient as cocaine, (2) apothesine is

practically equal to cocaine in anesthetic value. Sollmann's conclusions from his test by this method, namely, that novocaine is equal to cocaine, and apothesine one-eighth as efficient, are evidently incorrect. It is impossible to explain this discrepancy. Novocaine anesthesia is well known to be less permanent, since most observers have noted quick recovery. This, however, was not the factor in these tests, since the time of recovery was only incidental in importance as compared with the degree of anesthesia from the high dilutions.

In the introduction it was stated that since tests of local anesthetics by different methods do not show entire agreement, they can be taken as only suggesting and not demonstrating the clinical value. Viewed in another way, however, it may be said that these tests demonstrate comparative efficiencies in the particular method of applying the substance.

That both novocaine and apothesine are inferior to cocaine by surface application agrees with clinical evidence which has demonstrated cocaine to be exceptionally rapid in its absorption from mucous surfaces. Braun.⁴ The comparative efficiencies of cocaine and novocaine by intracutaneous injection also duplicates the opinion generally held that the latter is less efficient. On this point, Braun⁴ states: "Experience and experiments have, however, shown that by doubling the dose of novocaine so as to make it as effective as cocaine, and at the same time by adding certain substances which will be described in the next chapter (suprarenin), novocaine has become an ideal anesthetic for injection of tissues and has made cocaine unnecessary." It may be assumed therefore that this method correctly measures the efficiency of apothesine as well as those of cocaine and novocaine. Verification of the motor nerve method can not be cited at this time.

Based on the foregoing results it is evident that from every point of view apothesine is equal to or exceeds novocaine as a local anesthetic and in some respects is not less efficient than cocaine. We can therefore be assured of supplies of an efficient product, independent of German patents or controlled United States laboratories operating under license to German patentees.

The author's appreciation is extended to Dr. A. W. Lescossier, who carried out the intracutaneous tests and recorded his observations; also to three associates who by their courtesy made more data available.

TABLE IX.

SUBSTANCE	METHOD	GRAPHIC ILLUSTRATION OF EFFICIENCIES.	RATIO
COCAINE	Motor Nerve	=====	1
	Surface	-----	1
	Intracutaneous	+++++	1
NOVOCAINE	Motor Nerve	=====	1
	Surface	-----	1/3
	Intracutaneous	+++++	1/2
APOTHESINE	Motor Nerve	=====	1
	Surface	-----	1/3
	Intracutaneous	+++++	1

BIBLIOGRAPHY.

¹Sollmann: *Jour. Am. Med. Assn.*, 1918, vii, 216.

²Sollmann: *Jour. Pharm. and Exp. Therap.*, 1917, x, 379.

³Braun: *Local Anesthesia*, Translated by Shields, ed. 3, p. 80.

⁴Idem: p. 122.

⁵Gradenwitz: *Ber. klin. Wchenschr.*, 1899; xxxvi, 76.

**Studies from the Research Laboratory.
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**SOME STUDIES ON THE RESISTANCE OF THE OVA OF
TOXASCARIS LIMBATA.**

BY MEYER WIGDOR, A.M.

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The resistance of ascarid eggs to various chemical agents and to varying conditions of temperature, oxygen, and moisture has been known. Baillett (1866), referring to the resistance of ascarids, says that the shell of the egg "is so impermeable and resisting that it can only be affected by very energetic chemical agents, and in the majority of cases this shell is sufficient to protect the contents of the egg against everything that in ordinary circumstances might alter them." Verloren kept for more than a year ova of *Ascaris marginata* in which the embryos—formed from the fifteenth day—remained alive, although they had been exposed to all the variations of temperature during summer and winter. Braun (1906), in regard to *Ascaris canis*, states:

"The eggs, in spite of the delicacy of their shells, have great powers of resistance, and develop equally well in water and damp earth, in a solution of chromic acid, alcohol, turpentine, solution of soda, etc. It is, however, but seldom that the embryos hatch out."

Foster (1916), referring to the resistance of ascarid eggs, states:

"It is a well-known fact that, in the case of several species of parasites, the ova of which are characterized by a relatively thick egg shell, the eggs are affected but little, if at all, by formalin solutions. Ascarid eggs, for example, may be kept alive for months, or even years, in formalin."

Morris, when examining some human feces which contained many eggs of *Ascaris lumbricoides*, and which had been preserved in a 2-per-cent solution of formalin for two years, found that some of the eggs contained actively motile embryos. Four months

later there was an apparent increase in the number of eggs containing embryos. In my own experience, it has been found that a formalin solution is a very satisfactory medium in which to incubate ascarid eggs, as it prevents the growth of moulds, bacteria, etc., without interfering with the development of the embryos. Various other substances commonly destructive to protoplasm have been found not to interfere with the development of ascarid eggs. Leuckart notes that the eggs of *Ascaris mystax* may reach development in alcohol, chromic acid and turpentine; while Bataillon has had the ova of *Ascaris megalocephala* showing living embryos after having been for six months in Fleming's solution; he also finds that the embryos in the eggs remain intact and active in 50 per cent alcohol, in a 33 $\frac{1}{3}$ per cent solution of acetic acid, and in a 20 per cent sulphuric acid solution.

While the resistance of ascarid eggs is fairly well known in a general way, much work remains to be done in ascertaining substances which will destroy these and other helminth eggs. Since prophylaxis against parasitic infestation is largely a matter of proper disposal of manure or feces, a knowledge of suitable chemical agents for the destruction of the ova present in feces or manure is evidently desirable. It is known that, when live stock are pastured in the same field year after year, the animals often become unthrifty and occasionally sicken and die, due to their being continually in contact with soil polluted by parasitic ova and bacteria from manure. Just as there is an imminent danger of unthriftiness amongst animals in contact with polluted soil, similarly human health may be affected. Parasites of the intestine, lungs, liver, kidneys and bladder are usually spread by soil pollution. Whether these parasites have a simple life history without an intermediate host, or have an intermediate host, whether they spread from one person to another, from one of the lower animals to another, or infect one group after passage from another, the fact that the eggs are located in the feces for a time, and that here is an excellent opportunity to apply control measures, makes the study of means of attack against parasitic eggs an important and reliable investigation.

Just as various bacteria seem to show specific differences in their behavior toward certain chemicals, so may we expect the

same to be true in regard to the ova of parasites, and hence the need for further studies along these as yet almost untouched lines of investigation.

To prevent the evil effects of soil pollution from extending to his live stock, the farmer resorts to such measures as the purchase of additional pasture lands, pasture rotation, burning over of the pasture, etc. Human beings, on the other hand, are taught to frequent an appointed place to deposit excreta, which is then variously disposed of.

Several measures have been advocated for the treatment and disposal of manure and excreta, to kill parasitic ova, the commonest of which are:

1. *Heating.* Stiles and Lumsden claim that heating the effluent in a vessel at 212° F. is the only measure which can be unreservedly recommended to date. We would naturally expect that very high temperatures would prove lethal to parasitic ova due to the coagulation of the protoplasm. The disagreeableness of this procedure, however, is very evident, and is so considerable as to make this method impracticable.

2. *Burial.* Stiles and Lumsden state, concerning the method of disposal: "Burial will unquestionably decrease the danger of spreading infection, but in the present state of knowledge this method of disposal cannot be relied upon as safe." One danger involved is the probable contamination of water supplies.

3. *Chemical disinfection.* As has been previously stated, the chemical disinfection of polluted soil against parasitic worm eggs has received very little attention, although it appears to be a very feasible method of combating parasitic infection. Chemical disinfectants, such as chlorinated lime and certain coal-tar derivatives, have long been advocated to destroy parasitic bacteria in polluted soil, and there is no apparent reason why this method should not prove equally efficacious against eggs, if suitable substances can be found. There are certain factors to be taken into consideration, however, in chemical disinfection against eggs. Parasitic ova usually possess a strong chitinous outer membrane, which is lacking in bacteria, and which makes the egg very resistant to the penetration of most chemical agents. The usual germicidal strengths advocated to destroy bacteria prove surprisingly inadequate in destroying parasitic ova.

Another important factor to be considered in this connection is the effect of the chemical agent on the fertilizer value of feces. In many countries, as in China, where every bit of excreta is religiously kept and used, the effect that various chemicals would have on human feces in modifying its value as fertilizer is of prime importance. Another important factor already noted is that some parasitic ova are more resistant than others, and that the ova of different species may behave differently under the influence of various chemicals.

This paper is intended primarily to present a brief study of the effect of some chemical agents on the ova of one of the dog ascarids, *Toxascaris limbata*. The egg of this species was used because infested fecal material was readily available and because it seems quite resistant to chemical agents. The ova of *T. limbata* possess an outer, clear, double-contoured, chitinous shell and an inner yellowish membrane, which is marked with interlacing striations, giving the suggestion that this membrane is composed of interlaced fibers. The following procedure was usually employed in testing the various chemicals under consideration: The feces were collected, thoroughly broken up in a shaker and screened, a method advocated by Hall (1917) for examining feces. To most of the solutions, where it was feasible, a weak solution of approximately 5-per-cent potassium dichromate was added to hasten embryo development. Hall and Wigdor (1918) have found a 10-per-cent potassium dichromate solution a very satisfactory medium for culturing coccidia and various helminth ova, presumably by furnishing oxygen and hindering bacterial growth. Since in these tests the chemical agent was not applied to the feces direct, as under natural conditions, the results may differ somewhat from what may be actually found to take place if the feces were treated direct, without going through the screening process. Eggs that have been screened, however, should be more readily accessible to the chemical agent tested than those intact in the feces, for the latter have a coating of fecal matter protecting them to some extent against the agent employed, which protection is not afforded the screened eggs. The latter are mixed with, but not coated by, the fine particles of fecal matter which pass through the screen with the eggs. Since the tests were made on a parasite of the dog, the findings can only be applied to human.

parasites within certain limits and with some reservation, but that they will apply in large measure seems entirely reasonable and probable.

The chemical agents tested include the following groups of chemicals: (1) Acids, including hydrochloric, nitric, sulphuric, oxalic and acetic acids, and the alkalis, including caustic soda, ammonia and lime. (2) Metallic and other salts, including corrosive sublimate, copper sulphate, iron sulphate, potassium dichromate, potassium arsenite, sodium chloride and sodium fluoride. (3) Phenols, including pure carbolic acid, and kreso,* kreso dip,* septic,* cresylone* and neko,* preparations whose germicidal value depends on the higher phenols. (4) Alcohol. (5) Formaldehyde. (6) Volatile oils and other readily volatile agents, including chloroform, ether, oil of turpentine, oil of chenopodium, toluol and xylol. (7) Miscellaneous agents, including hydrogen peroxide and germ-X.†

Additional tests were made to determine the rate of development, if any, of screened feces in distilled water, of screened and unscreened feces in tap-water, and of screened and unscreened feces in tap-water to which some 5-per-cent potassium dichromate was added. Tests were also made on the effect of temperature and of moisture on egg development.

The number of chemical disinfectants is large, and nearly every group of chemical substances includes members that may be capable of injuring parasitic ova. In practice, however, only a relatively small number of chemicals come under consideration, it being necessary to exclude all that act only in a high state of concentration, as well as those which unduly corrode containers to be disinfected or which are too expensive.

The number of chemical disinfectants that might be employed in attempting to destroy parasitic ova has by no means been exhausted in these studies, but some of the most important members of each group have been tested and will be given *seriatim*.

*These phenol preparations are marketed by Parke, Davis & Co. under these trade names, and will hereafter be referred to as Preparations *A* (Kreso), *B* (Kreso Dip), *C* (Septico), *D* (Cresylone), and *E* (Neko).

†Germ-X, a hypochlorite, will be termed Preparation "X," and is marketed by the North Star Chemical Works, of Lawrence, Mass. Similar products on the market are Bacilli Kill and Fecto.

ACIDS AND ALKALIS.

Since the development of ova is dependent on certain chemical reactions, varying between somewhat narrow limits, all strong acids and alkalis adversely affect the vital processes. Certain acids, such as hydrochloric acid, also sulphuric and nitric acids, have long been known to kill all germs in a very short time, while the antiseptic value of other acids, such as acetic, is only slight. Their highly destructive action on most objects, however, stands in the way of their employment in practice, and consequently the cheapest of the strong acids—hydrochloric acid and sulphuric acid—are rarely used.

Regarding the resistance of parasitic ova to various acids, there is very little available data. Wharton (1915) found that eggs of *Ascaris lumbricoides* died in one-half per cent hydrochloric and 3 per cent acetic, and divided overnight in 3 per cent nitric. Bataillon (1901), as cited by Foster (1916), found, on the other hand, that the embryos in the eggs of *Ascaris megalocephala* remained intact and active in a 33 $\frac{1}{3}$ per cent solution of acetic acid and a 20 per cent solution of sulphuric acid.

Hydrochloric, sulphuric, nitric, oxalic and acetic acids were the acids used in these tests, with the following results:

At the end of three days, eggs kept in a 5 per cent hydrochloric acid solution showed motile embryos. Those in a 10 per cent solution, for the same period of time, showed embryo development, but the embryos were apparently immotile.

Eggs kept in a 5 per cent sulphuric acid solution showed some actively motile embryos at the end of a period of three days. Most of the eggs, however, were still segmenting and dividing, and all were in apparently good condition. Eggs kept in a 10 per cent sulphuric acid solution for three days showed embryo development in some cases, but the embryos were apparently immotile.

Eggs kept in a 5 per cent solution of nitric acid showed some actively motile embryos at the end of a three-day period. About 50 per cent of the eggs were, however, killed and shrunken to a little less than one-half their normal size. The other 50 per cent were in good condition and were undergoing development.

Eggs kept in a 20, 30, 40 and 50 per cent solution of oxalic acid showed actively motile embryos in three days.

Eggs kept in 5, 10 and 20 per cent solutions of acetic acid for three days showed actively motile embryos at the end of that period. Eggs kept in a 30 per cent solution for the same period were killed, and had not undergone any marked development.

Thus, amongst the acids, nitric acid appears to be the strongest in its ovacidal action against the eggs of *Toxascaris limbata*, sulphuric and hydrochloric of approximately equal ovacidal strength ranking next, acetic next, and oxalic last.

The action of alkalis is, broadly speaking, less powerful than that of the acids. Caustic soda is held to be the strongest of these agents. In actual practice, the strong alkalis will only occasionally come into consideration for use as disinfectants, since they corrode most articles that require treatment. An exception is, however, afforded in the case of slaked lime, which is extensively used in practical disinfection. The treatment of manure with slaked lime has been perhaps the most widely advocated measure for combating parasitic infection.

To determine the efficacy of this treatment in destroying the ova of *T. limbata*, the following tests were made:

Five grams of feces were placed in a petri dish and 0.45 gram of slaked lime sprinkled thereon. Ten days later embryos were found to have developed, but they were apparently immotile.

Eggs were placed in a one-fifteenth solution (1 gm. of the lime to 15 gms. of water) of slaked lime; embryo development was noted three days later.

Thus it appears that the treatment of the feces with the ordinary commercial slaked lime does not hinder the development of the ova of *T. limbata*. In this connection it must be taken into consideration that slaked lime, to be at all effective, should be freshly slaked, for, on being exposed to the air, it is readily converted into the carbonate form, which is devoid of antiseptic properties. Commercial preparations of slaked lime are very often not fresh-slaked lime, but are the carbonate, and hence of no value for disinfection.

Embryo development was also obtained in three days in eggs kept in a one-fifteenth solution of chlorinated lime.

The action of caustic soda on the ova of *T. limbata* is of special interest, however. Eggs were cultured in 1, 2, 5, 10 and

50 per cent solutions of caustic soda, and motile embryos were obtained in each case at the end of a three-day period. Some of the eggs in the 10 and 50 per cent solutions were, however, undergoing decomposition. In a 25 per cent solution all the eggs were killed and showed degeneration. The resistance of the ova to caustic soda is surprising, in view of the latter's well-known disintegrating action on chitin. In 50 per cent strengths a protective coat appears to be cast about the egg, making it impermeable to the action of the alkali.

Ammonium hydroxide appears to be devoid of any action against the ova, since motile embryos were obtained at the end of a three-day period in 25 and 50 per cent solutions of the alkali.

From the above we can note that the ova of *T. limbata* show surprising resistance to the action of most of the common acids and alkalis.

METALLIC SALTS AND OTHER SALTS.

The group of metallic salts is of considerable importance, and comprises some of the most powerful disinfectants against bacteria known.

Corrosive sublimate (mercury dichloride Hg Cl_2) is known to destroy the vegetative forms of bacteria in a few minutes, even when diluted to 1 part in 10,000; and the spores of bacteria possessing medium powers of resistance, such as anthrax spores, are killed within two hours by a 1-1000 solution. However, like many other disinfectants, all metallic salts are influenced by other substances present in the solution, and also by the solvent, because their disinfectant power depends on their degree of electrolytic dissociation. For this reason they act much less powerfully in an alcoholic solution, and not at all in a fatty or oily medium. The dissociation may be also modified by additions of other agents.

Tests on various salts of this group gave the following results:

Eggs kept in a 1-500 solution of corrosive sublimate showed motile embryos at the end of a three-day period. Eggs kept in a 1-250 solution of the salt showed division at the end of three days, a high degree of segmentation at the end of five days and actively motile embryos at the end of fifteen days. Eggs in a 1-100 solution showed actively motile embryos in three days.

Eggs kept in a 20 per cent copper sulphate solution showed actively motile embryos at the end of three days.

Eggs kept in a 33 per cent solution of iron sulphate showed actively motile embryos at the end of three days.

Eggs kept in normal saline solution showed actively motile embryos at the end of three days.

Eggs kept in 1-2 solution of sodium fluoride, a salt which has recently been advocated as possessing valuable antiseptic properties, showed actively motile embryos at the end of three days.

Eggs kept in a 1 per cent solution of potassium arsenite showed actively motile embryos at the end of three days, but they were apparently dead when examined two days later. Eggs kept in a 10 per cent solution of potassium arsenite showed some actively motile embryos at the end of three days. Some of the embryos were apparently immotile, and some of the eggs had apparently undergone very little development, for the nuclear material in the eggs was distorted and was breaking down.

In this connection it may be stated that a 10 per cent potassium dichromate solution has proven a very valuable medium for developing not only the eggs of *Toxascaris limbata*, but also the eggs of *Ancylostomum caninum*, *Trichuris depressiuscula* and the oocysts of *Diplospora bigemina*.

The ova of *T. limbata* are, therefore, highly resistant to the action of most metallic salts which have been known to possess bactericidal properties.

PHENOLS.

This generic term includes all the chemicals allied to true phenol (carbolic acid), which form a very important group of disinfectants.

Carbolic acid is soluble to the extent of 5-6 per cent in water, and when employed for disinfection purposes is usually replaced by its homologues, the cresols and their compounds, which are cheaper and less corrosive. The three cresols, meta-, para- and orthocresol, are in themselves too sparingly soluble (0.5, 1.8 and 2.5 per cent, respectively) to exert any powerful disinfectant action, but their solubility can be largely increased by the addition of strong acids or of alkaline soaps, which raise them to the category of the strongest disinfectants. The greatest popularity is

enjoyed by the cresols which have been dissociated by means of soap solutions, and which fall into two categories, one class forming clear solutions in water and the other an emulsion in water. Of the latter, preparations *A**, *B** and *E** and of the former, preparations *C** and *D** are representatives which have been tested. Preparation *B*, with a phenol coefficient of 5, consists of 78 per cent creosote oil and 23 per cent resin soap, with enough water added to keep it in solution. Preparation *A*, with a phenol coefficient of 6 or 7, consists of 70 per cent creosote oil enriched with extra phenols and 30 per cent soap solution. Preparation *D*, with a phenol coefficient of 2, consists of a 50 per cent solution of cresylic acid in soap and water. Preparation *E*, with a phenol coefficient of 16 to 20, consists of 78 per cent high coefficient oil, which has a higher percentage (about 90 per cent) and higher quality of phenols than the ordinary coke-oven tar phenols. Preparation *C*, with a phenol coefficient of 2, is almost identical with preparation *D*, differing in that it contains about 10 per cent of oils (eucalyptus, camphor and turpentine oils) to give it a pleasant odor.

The following results were obtained on the resistance of the ova of *T. limbata* to phenol and its derivatives. Tests on pure carbolic acid were made as follows:

Eggs placed in pure carbolic, full strength, were killed at the end of three days. The eggs were greatly distorted and had shrunk to about one-half their normal size. The shells were split at several points and the eggs were undergoing degeneration.

Eggs in 20 per cent and 5 per cent carbolic were found dead at the end of a three-day period. The eggs were deformed.

Eggs in a 2 per cent carbolic acid solution showed no development at the end of a three day period, but the eggs were fairly well preserved.

Eggs in a 1 per cent carbolic acid solution showed division and segmentation at the end of three days, and at the end of five days no further development was noted, the eggs being apparently killed.

*Presentations by J. H. H. van den Kerkhof and K. van Kesteren, Kees-Dirk, Septicon, Cresylone and Neolene, respectively.

Tests on preparation *B* were made as follows:

Eggs kept in 1-500 solution showed actively motile embryos in three days.

Eggs kept in 1-250 solution showed motile embryos in three days, but most of the eggs had not yet developed to form embryos, being still in the division stage. On the twelfth day after culturing the embryos were found dead and undergoing degeneration.

Eggs in a 1-50 and 1-100 solution, at the end of three days were, in nearly all cases, undergoing complete degeneration, the chitinous outer membrane and nuclear material being almost entirely destroyed. Some eggs had undergone embryo development, but had been killed and were breaking down.

The advocated disinfectant strength of preparation *B* is 1 part of the preparation to 100 parts of water, a strength which proved entirely efficacious in destroying the ova of *T. limbata*.

Tests on preparation *A* were made as follows:

Eggs kept in a 1-250 solution showed embryo development at the end of three days, but the embryos were apparently dead and the nuclear material was breaking down.

Eggs kept in a 1-100 solution showed division, segmentation and some embryo development at the end of three days. At the end of five days the eggs were dead and the nuclear material was decomposing.

Eggs kept in a 1-50 solution were nearly all dead at the end of three days; some of the eggs were highly segmented, but apparently dead.

The advocated disinfectant strength of preparation *A* is 1-100.

Tests on preparation *D* were made as follows:

Eggs kept in a 1-250 solution for three days showed motile embryos at the end of that period.

Eggs kept in a 1-100 solution showed slow development at the end of a four-day period. The eggs were segmented in most cases and the young embryos were just ready to appear.

Eggs kept in a 1-50 solution for four days showed no noticeable development at the end of that period, all the eggs being apparently dead.

The advocated disinfectant strength of preparation *D* is a 1

or 2 per cent solution. In these tests a 2 per cent strength seems to be effective against the ova of *T. limbata*.

Tests on preparation *C* were made as follows:

Eggs kept in a 1-15 solution showed embryo development at the end of a three-day period.

Eggs kept in a 1-50 solution showed little development at the end of a three-day period, although they were all apparently in good condition, some showing the beginning of segmentation.

Eggs kept in a 1-25 solution were killed at the end of a three-day period and were breaking down.

Preparation *C* is advocated in strengths of 1-15 for spraying barns, stables, etc., and in a solution of 2 per cent strength for sterilizing wounds. These tests have shown that a 1-50 solution will apparently kill and a 1-25 solution will surely kill.

Tests were made on preparation *E* as follows:

Eggs kept in a 1-500 solution showed actively motile embryos at the end of three days.

Eggs kept in a 1-250 solution showed a few actively motile embryos at the end of a three-day period, but most of the eggs were apparently killed. At the end of four days the embryos were apparently dead.

Eggs kept in a 1-100 solution showed very little development and were apparently dead after a period of three days.

The dilution of preparation *E* recommended for general use is 1-500. The tests on the ova of *T. limbata* proved this strength to be inadequate for inhibiting embryo development. A 1-250 or, still better, a 1-100 solution, is advisable.

The above data on the action of the phenols show that there is a direct relationship between the corrosiveness of the phenol used and its ovacidal action against the ova of *T. limbata*. Pure phenol, highly corrosive, kills the eggs in a 1 per cent solution; preparations *C* and *D*, the next most highly corrosive substances used, kill in solutions which are equivalent to a 4 per cent solution of phenol; preparations *A* and *B*, ranking next in their corrosive action, kill in a solution which is equivalent to a 7 per cent solution of phenol; and preparation *E*, the least corrosive and hence least efficacious against the ova tested, kills in solutions which are equivalent to a 10 to 20 per cent solution of phenol.

This group offers the most promising possibilities for destroying parasitic ova. Strong solutions of such phenols as preparations *A*, *B*, *C* and *D* should prove highly effective in killing worm ova.

ALCOHOL.

The alcohols are still the subject of scientific discussion, in so far as their disinfectant properties are concerned. That they are endowed with a by no means small power of disinfection is indubitable, but the scientific experiments performed in this connection have furnished widely different results in detail. On the whole, it has been ascertained by careful research that solutions above 20 per cent in strength kill all vegetative forms of moist and dried bacteria, and that this action increases in power up to solutions of 80 per cent strength, beyond which limit it declines in the case of dried bacteria, but persists through the higher strengths (85, 90) in the case of moist bacteria. In my tests, actively motile embryos were present in the eggs after a three-day period in solutions of 10, 25, 50, 60 and 70 per cent strengths of ethyl alcohol, but were killed in 75 per cent and higher strengths, thus agreeing with the results obtained for the action of ethyl alcohol on moist bacteria. The eggs in the latter solutions had apparently lost their inner coat or it had been rendered homogeneous and invisible.

It is interesting to note that Bataillon, as previously mentioned (1901), found that the embryos in the eggs of *Ascaris megalocephala* remained intact and active in 50 per cent alcohol.

The ova of *T. limbata* are, therefore, very highly resistant to the action of alcohol.

FORMALDEHYDE.

The chief action of formaldehyde for bacterial disinfection is to restrict the growth of bacteria, which are prevented from germinating by solutions as weak as 1:20,000. In its ovacidal action against the eggs of *T. limbata*, formaldehyde is practically negligible. In this series of experiments eggs were cultured in 1, 5, 10, 20, 25, 30 and 35 per cent solutions of formaldehyde, and at the end of three days motile embryos were noted in every case.

Eggs cultured in commercial formaldehyde (an approximate 40 per cent solution) showed immotile embryos in a good many of the cases ten days later, while most of the eggs were still undivided and apparently in a state of preservation. As has been previously stated, Foster (1916) notes that ascarid eggs could be kept alive for months, even years, in formalin, and that it is a very satisfactory medium in which to incubate ascarid eggs. Foster (1916) cites Morris (1911), who kept some feces containing eggs of *Ascaris lumbricoides* in a 2 per cent solution of formalin for two years, at the end of which time he found some of the eggs contained actively motile embryos.

VOLATILE OILS AND OTHER READILY VOLATILE AGENTS.

This category comprises a number of substances belonging to a variety of chemical groups, and having in common the property of being only sparingly soluble in water and remaining solid or liquid at ordinary temperatures, but volatilizing readily. The chief substances of this group that were tested are: chloroform, ether, oil of chenopodium, oil of turpentine, toluol and xylol.

Chloroform: Eggs kept in this medium were found dead at the end of three days. The eggs were well cleared, the nuclear membrane was well outlined and the nuclear material within was very much cleared. The inner membrane was invisible.

Ethyl Ether: Eggs in this medium were found dead at the end of three days.

Oil of Chenopodium: Eggs in this medium at the end of three days showed no development, being apparently preserved. At the end of five days some eggs showed signs of division, but most of the eggs were distorted and apparently dead. At the end of seven days the eggs were very clear, and were breaking down and shrinking decidedly.

Oil of Turpentine: Eggs reared in this medium showed motile embryos in a great many cases at the end of five days. A great many of the eggs, however, were deformed, being flattened on one side and showing very little development. At the end of seven days the embryos that had developed were dead, most of the eggs being flattened on one side and decomposing.

Toluol: Eggs in this medium showed embryo development in

one or two cases at the end of three days, but nearly all the eggs were cleared and were undergoing degeneration. Where embryo development was noted, the embryos were in a poor state of preservation, being immotile and breaking down.

Nylol: Eggs in this medium showed embryo development at the end of three days, but the embryos were apparently dead.

The ova of *T. limbata* thus do not appear to be very resistant to the action of the volatile agents used.

MISCELLANEOUS AGENTS.

This group comprises hydrogen peroxide, which is known to possess very powerful bactericidal properties, due to its oxidizing effect on organic matter (diluted to 0.015 per cent it destroys all vegetative forms in a few minutes), and preparation X,* a hypochlorite (a mixture of sodium hypochlorite, sodium chloride, calcium chloride, calcium hypochlorite, made alkaline with lime water and containing 3 to 4 per cent of available chlorine).

Eggs kept in hydrogen peroxide (commercial 3 per cent solution) showed some motile embryos at the end of six days, but most of the eggs were undivided and apparently killed.

Eggs kept in a full-strength solution of germ-X, which has a phenol coefficient of 10+ and which is advocated in strengths of one fluid ounce (two tablespoonfuls) to one or two gallons, showed motile embryos at the end of three days.

Thus, hydrogen peroxide and a hypochlorite, both widely used in bactericidal disinfection with much success, were both ineffective against the ova of *T. limbata* in much more concentrated strengths than those advocated for bacterial disinfection.

EFFECTS OF MOISTURE, TEMPERATURE, ETC., ON THE RATE OF DEVELOPMENT OF THE OVA OF *TOXASCARIS LIMBATA*.

To determine the effect of lack of moisture on the development of the ova of *T. limbata*, some feces were screened and then spread over filter paper and allowed to dry at room temperature (24 to 29.5° C.). Three days after the feces were thus treated, actively motile embryos were found. The lack of moisture thus apparently seemed to hasten embryo development of the ova.

* Germ-X.

To determine the effects of temperature on the rate of development of the ova, eggs were screened and cultured in water to which 5 per cent potassium dichromate had been added. One culture was placed in the incubator and kept at a temperature of 37.8° C., another in an oven at a temperature of 49 to 60° C. for twenty hours, and the other placed in the refrigerator at a temperature of 10° C. At the end of three days, motile embryos were found in the eggs kept in the incubator, those kept in the oven were dead, while those in the refrigerator showed very little development. At the end of eight days the eggs in the refrigerator were showing division, and at the end of fifteen days were very highly segmented. At the end of twenty-eight days the eggs were still highly segmented, but no embryos were found, and at the end of thirty-eight days actively motile embryos were found.

Low temperatures thus retard the development of the ova of *T. limbata* (thirty-eight days for embryo development at 10° C.); room temperatures, 21 to 33° C., are very favorable for their development, while temperatures of 49 to 68° C. for several hours apparently kill. It is interesting to note that Wharton (1915) finds that the optimum temperature for the development of the ova of *Ascaris lumbricoides* is about 30° C., and that at the temperature of 37° C. the ova are killed after some time, and above this temperature they die rapidly. We also find that low temperatures retard the development of the ova of this species without killing. He further states that pig and calf ascarid eggs must be completely developed before exposure to a temperature of 37° C. or they die, while horse and dog ascarid ova develop at this temperature.

To determine the effect of the oxygen supply and bacterial action on the development of the ova of *T. limbata*, the following tests were made: Unscreened feces containing ova were placed in tap-water and at the end of eighteen days all the eggs were apparently dead, the nuclear material showing signs of decomposition.

Screened ova were placed in tap-water, and at the end of thirty days very little development was noticeable, most of the eggs undergoing degeneration.

Screened ova were placed in distilled water, and at the end of fifteen days motile embryos in good condition were found.

Screened and unscreened ova were placed in tap-water with 10 per cent potassium dichromate added, and at the end of three days actively motile embryos were noted.

The failure to obtain development in the first two cases (screened and unscreened feces in tap-water) is probably due to bacterial and other growths in the culture which utilize a large amount of the available oxygen, or even may excrete toxins detrimental to the development of the ova. In the latter cases, where the possibility of bacterial growth is reduced to a minimum and thus more available oxygen supplied to the ova, development is hastened. It is interesting to note that Wharton (1915) found that the eggs of *Ascaris lumbricoides* developed rapidly in tap-water and irregularly or died in distilled water. His results are just the reverse of those that I have obtained with the ova of *T. limbata*.

It is also interesting to note the results of the experiments of Stiles and of Stiles and Gardner (1911) on the fermentation in water of the eggs of *Ascaris lumbricoides* and *Necator americanus*. Fecal material kept in water and examined after 68, 117, 144, 317, 232, 349, 357 and 358 days showed all the hookworm eggs identified were dead. The longest period of time after which they were able to find live hookworm (*Necator americanus*) eggs under those conditions was seventy days. The longest period after which they were able to find *Ascaris lumbricoides* eggs was 117 to 121 days. Fermentation for four months in an L. R. S. privy is, therefore, advocated for killing all the hookworm eggs, and fermentation for three months for killing nearly all, probably all, the hookworm eggs.

It is held that, apart from the question of concentration, the action of an ovacidal agent depends, biologically, on the resistance of the ova and on the temperature; and, physically, on the capacity of the articles under examination to absorb moisture. The concentration of the ovacide is held to stand in direct relation to its action, within certain limits, but if the concentration be very high the action is only slightly increased, whereas, conversely, extreme dilution weakens the effect but slowly--though this is not always true, the effect alternating rapidly, up or down, when the concentration is modified. The resisting power of parasitic ova has already been mentioned, and, so far as the tem-

perature is concerned, its influence is based on the physiologically vital processes of the ova. An organism which is cooled below its optimum temperature gradually passes into a state in which the processes of nutrition and development are almost entirely suspended, according to the degree of cooling given. In this condition the ova have a corresponding low tendency to undergo chemical changes, and the ovacidal effect diminishes in intensity. On the other hand, the cell is far more open to chemical attack at its optimum temperature, at which all the vital processes, and therefore all the chemical reactions, go on best, while, as the temperature is raised above this point, the tendency of the ova to decompose increases, and they fall an easier prey to the destructive action of poison. Hence, the action of ovacidal agents is facilitated by a rise in temperature. Since the eggs in these experiments were cultured at room temperatures which varied between 21° C. and 33.2° C., the higher range of temperatures persisting over night (an interval of fifteen hours), the various chemical agents were given optimum conditions under which to operate.

SUMMARY.

Parasitic ova are very resistant to various chemical disinfectants.

The usually advocated germicidal strengths are markedly effective against the ova of *Toxascaris limbata* for many substances.

The ova of *T. limbata* show surprising resistance toward acids, alkalis (especially against caustic soda and lime) and metallic salts.

Ethyl alcohol in strengths up to 70 per cent and formaldehyde in varying strengths up to approximately 40 per cent are remarkably ineffective in their ovacidal action against the ova of *T. limbata*.

The phenol derivatives, primarily the cresols which have been dissociated by means of soap solutions, such as preparations A, B, C, D and E (varying in their lethal action on parasitic ova according to their corrosiveness), offer the best possibilities as ovacides against parasitic ova of all substances tested.

Most of the volatile disinfectants are apparently efficacious in killing the ova of *T. limbata*.

The ova of *T. limbata* are evidently very resistant to conditions of drought and to low temperatures, and require an ample supply of oxygen for the best development. Rapid development is possible at temperatures as high as 37.8° C., but the ova are killed at temperatures of 49 to 60° C., and development is materially retarded at temperatures as low as 10° C.

The writer wishes to take this occasion to very gratefully acknowledge the invaluable advice and assistance of Dr. M. C. Hall, through whose instigation this work was conducted.

BIBLIOGRAPHY.

- Baillet, C., 1866. Art. Helminthes. Nouv. Dict. Pract. de Méd., de Chir., et d'Hyg. Vétérinaire, VIII.
- Braun, M., 1908. Die tierischen Parasiten des Menschen, 4 Aufl. 8 vo. Wursburg Kabitzsch (IX+623 p. ...). Engl. Trans. of 3d ed., with additions by Sambon and Theobald, p. 337.
- Foster, W. D., 1916. A further note on polyradiate cestodes. *Science*, n. s., Vol. XLIV, No. 1333, 388-389.
- Hall, M. C., 1917. Apparatus for use in examining feces for evidences of parasitism. *Jour. Lab. and Clin. Med.*, V. 2 (5), February, 347-353, 3 figs.
- Hall, M. C., and M. Wigdor, 1918. Canine coccidiosis, with a note regarding other protozoan parasites from the dog. *J. A. V. M. A.*, 6 (1), April, 64-76, 1 fig.
- Stiles, C. W., and L. L. Lumsden, 1911. The Sanitary Privy. *Farmers' Bull.*, 463, U. S. Dept. Agr., 1-32.
- Stiles, C. W., and H. C. Miller, 1911. Observations on the Viability of the Eggs of Hookworms (*Necator americanus*) and of Eel-worms (*Ascaris lumbricoides*) in Feces Allowed to Decompose in Water. *Pub. Health Reports*, XXVI, No. 41, 1565-1567.
- Wharton, L., 1915. The Development of the Eggs of *Ascaris lumbricoides*. *Phil. J. Sci.*, V. 10 (1), January, pp. 19-23.

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A NEW FLUKE FROM THE DOG.

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Recently Hall and Wigdor (1918) reported the occurrence of two new flukes, *Alaria americana* and *Alaria michiganensis*, in Detroit dogs, which were the first authentic cases of intestinal fluke infestation of dogs in North America. In fact, the only fluke that appears to have been reported from dogs in the United States is *Paragonimus kellicotti*, which occurs as a pulmonary parasite of dogs, cats and swine.

In our series of 350 dogs examined postmortem at Detroit, intestinal flukes were found in 8 animals, *A. americana* and *A. michiganensis* being represented in 7 of these and a new, heretofore undescribed, species (12 specimens) in the other.

An examination of this new species of fluke shows that it falls into the sub-family Opisthorchiinae, but it cannot be correlated with any well-established genus within that group, and hence has been placed in a new genus, *Hallum*, Wigdor, 1918.

This genus appears to fill a gap in the genera of the sub-family Opisthorchiinae as regards the extent of the vitellaria. In the genus *Opisthorchis*, the vitellaria are confined in the area posterior to the acetabulum and anterior to the ovary and testes; in the genus *Amphimerus* the vitellaria do not extend anteriorly beyond the acetabulum and frequently extend posteriorly to or beyond the posterior testis; in the genus *Metorchis* the vitellaria extend anteriorly beyond the acetabulum and do not extend beyond the ovary and testes; in the genus *Hallum*, however, the vitellaria extend anteriorly beyond the acetabulum and posteriorly beyond the ovary and testes.

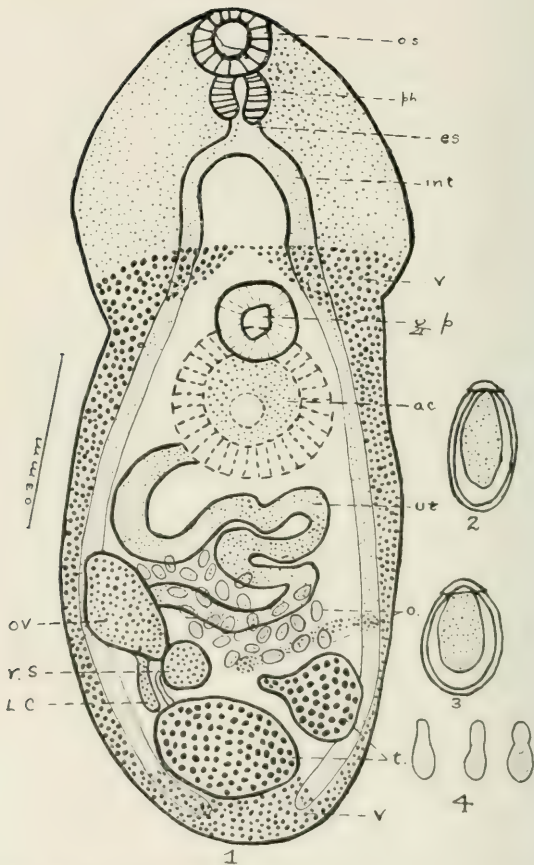


Fig. 1. ANTERIOR PART OF THE BODY OF A NEMATODE, DORSAL VIEW.

os — oral sucker; ph — pharynx; es — esophagus; int — intestine;
v — vagina; x p — X-plate; ac — acetabulum; ut — uterus;
o — ovary; r. s. — receptaculum seminis; L C — liver cells.

Fig. 2. Egg of the nematode.

Fig. 3. Cluster of eggs of the nematode.

Fig. 4. Sperm cells of the nematode.

Members of the sub-family Opisthorchiinae have been reported as occurring in the bile ducts or gall bladder of man, mammals, birds, reptiles and fish, this being the first report of the occurrence of one of the species of this group as an intestinal parasite.

SUB-FAMILY OPISTHORCHIINAE, LOOSS, 1899.

Sub-family diagnosis—Fasciolidae of medium size, with slender elongated body, noticeably tapering anteriorly. Suckers near each other and generally not strongly developed. Pharynx present. Esophagus short and slender, intestinal ceca long and simple. Excretory system Y-shape, arms short, main stem long and S-shape, winding between the testes. Genital pore median and anterior to the acetabulum. Copulatory organs present. Testes close together in the posterior end, the one more or less obliquely behind the other. Laurer's canal present. Receptaculum seminis very strongly developed. Uterine coils anterior to the ovary. Vitellaria strongly developed, lateral of the intestinal ceca.

GENUS HALLUM, WIGDOR, 1918.

Generic diagnosis—Thin, flattened, transparent forms, with body often attenuated at anterior extremity and a posterior broader end; anterior end sometimes constricted at the level of the acetabulum; anterior extremity frequently covered with small, retrose spinelets. Suckers quite widely separated. Acetabulum larger than the oral sucker. Digestive tract with distinct muscular pharynx, short esophagus and two long simple intestinal ceca. Genital pore median, immediately anterior to the acetabulum. Copulatory organs absent. Testes in posterior portion of the body, simple or lobate, the one obliquely posterior to the other. Ovary slightly anterior to testes, either simple or slightly lobed. Laurer's canal and receptaculum seminis present. Uterine coils anterior to ovary and generally do not extend over the intestinal ceca. Vitellaria in one region well-developed, extending cephalad beyond the acetabulum and posteriad beyond the posterior testis; vitellaria lateral of the intestinal ceca in post-acetabular region and often extending median in preacetabular region.

Type species—*Hallum caninum*, Wigdor, 1918.

SPECIES HALLUM CANINUM, WIGDOR, 1918.

Specific diagnosis.—Body flat and transparent; anterior portion bluish and posterior whitish; anterior portion either attenuated or constricted in the region of the acetabulum. Anterior portion of body usually covered with small retrose spinelets. Length of body 0.902–1.642 mm. Width at widest part 0.131–0.170 mm. Oral sucker sub-terminal 0.066–0.104 mm. in diameter. Length of pharynx 0.066–0.080 mm. Esophagus very short, 0.040–0.060 mm. long. Intestinal ceca usually equal, extending to the posterior end of the body. Acetabulum much larger than oral sucker, measuring 0.136–0.184 mm. in diameter, and situated somewhat anterior of the middle of the body. Genital pore prominent, median, at anterior margin of the acetabulum. Ovary usually slightly anterior to testes, oval or elliptical in shape and usually smooth in outline. Cirrus pouch absent. Testes approximately in posterior eighth of body, one obliquely behind the other, usually orbicular or oval in shape and either smooth or lobed in outline. Uterine coils well developed, filling a good portion of the body between the intestinal ceca, the ovary and the acetabulum, the coils not extending over the intestinal ceca. Laurer's canal and receptaculum seminis present, but usually not very prominent. Vitellaria well developed, extending 0.120–0.220 mm. anterior of the acetabulum and posterior beyond the posterior testis, filling up most of the posterior portion of the body posterior of the testes. The vitellaria usually extend laterad of the intestinal ceca in the post-acetabular portion of the body, but usually extend over the intestinal ceca to the middle of the body anterior of the acetabulum. Eggs reddish brown, with a distinct lid and opercular rim, measuring 0.032–0.048 mm. by 0.018–0.022 mm.

Host.—*Canis familiaris*.

Location.—Small intestine.

Locality.—Detroit, Mich.

SHERR, Frederick H., 1922. The *Trinematode genus Canis* (Schrank). Arch. de. Parasit., 5: 144 et seq. (in part).

WIGDOR, C. and M. WIGDOR, 1918. *Trinematode parasites of the dog*. Jour. Nat. Hist. N. York, N. S., Vol. 50, no. 3, pp. 616–626, 3 figs.

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**A NOTE ON THE EFFECT OF COLD ON THE DEGREE
OF PARASITIC INFESTATION.**

MEYER WIGDOR, M.A.

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In our anthelmintic investigations conducted throughout the winter of 1916 and up through January, 1918, about 300 Detroit city pound dogs, which were fairly representative of almost all breeds of dogs, except the toy varieties, were used. During this period suitable infested animals for anthelmintic treatment were readily obtainable, for a very large percentage of the dogs were infested with internal parasites.

Of close to 400 dogs that were examined, about 100, or 25 per cent, were rejected for experimental purposes on the strength of a negative fecal examination, which does not, however, prove that they were not infested with intestinal parasites. There may be worms present in spite of the absence of ova in the feces, for the worms might be all males, or the females might be immature or so few in number that egg production is very limited, and hence undetected, or egg production might be inhibited by several factors. Dogs which show negative fecal findings on examination, when examined postmortem, are found to be infested. Of the 300 dogs examined postmortem, 271, or 93 per cent, were found infested with intestinal parasites. Thus, 271 dogs out of 400, or about 68 per cent of the Detroit dogs examined, were surely infested, and since some of the 100 dogs that were rejected would be found on postmortem examination to be infested, it can safely be asserted that more than seven out of ten Detroit dogs, under normal conditions, are infested with intestinal parasites.

Detroit dogs are infested with two species of ascarids, *Belascaris marginata* and *Toxascaris limbata*. Of the two species,

Belascaris marginata was apparently the commoner one met with in our first series of 300 dogs. Of our first 67 infested, 47, or 70 per cent, were infested with ascarids, and, according to Hall (1917), all that were examined proved to be *Belascaris marginata*. Of the 271 in the series of 300 infested, 144, or 53 per cent, were infested with ascarids, a large number of which proved to be *T. limbata*.

Next to the ascarids in the frequency of nematode infestations are the whipworm, *Trichuris depressiuscula*, which occurred in 111 of our 271 infested dogs, or in 41 per cent, and the hookworm, *Ancylostoma caninum*, which occurred in 89 of our 271 infested dogs, or in 33 per cent.

Thus, with 53 per cent of our Detroit dogs infested with ascarids, 41 per cent with whips, and 33 per cent with hookworms, very suitable material for anthelmintic investigations was always at hand. During the last few months, however—that is, during the latter part of February, March, April and May, 1918—approximately 50 out of 60 dogs examined showed negative fecal examinations with an almost total absence of hookworm infestation. Furthermore, the species of ascarid met with was not the expected common *B. marginata*, but the rarer of the two ascarid species, *T. limbata*. This marked falling off of parasitic infestation, as evidenced by negative fecal examinations, which is not, as has been stated before, entirely conclusive evidence of the absence of intestinal parasitism, is apparently due to the unusually prolonged, severe winter experienced this last year (1917-1918). It has been known that cold will retard the development of parasitic ova, and Stiles (1908) in regard to the effect of temperature on the hookworm eggs of man says: "Cold retards and heat hastens the development of the eggs and embryos; a freezing temperature of 24 to 48 hours' duration, it is said, kills both eggs and embryos." During this past winter, when the thermometers about Detroit registered as low as -20° and remained around zero for several days, the opportunity for development of most parasitic ova was reduced to a minimum.

The greater frequency of *T. limbata* in our dogs is apparently due to the greater resistance of its ova to extreme temperatures. The ova of *T. limbata* are provided with a double contoured chitinous shell and an inner coat marked with interlacing stria-

tions suggesting fibres, which apparently affords the egg great protection against low temperatures. I have obtained embryo development in the ova of *T. limbata* in 35 days at a temperature of 10° C. At room temperatures, 21-33° C., embryo development was noted in 2 to 3 days. Thus, temperatures as low as 10° C. merely tend to retard the development of the ova of *T. limbata*. Ova such as those of *B. marginata* and *Ancylostoma caninum*, which have no such highly developed shell, are undoubtedly unable to withstand the vicissitudes of low temperatures for any length of time.

It is interesting to note the results of the frequencies of parasitic infestations of dogs in regions south of Detroit, where warmer climate would evidently promote the development of parasitic ova, and hence the degree of parasitic infestation.

Hall (1917) found that of 76 infested dogs examined at Washington, D. C., 67 per cent of the dogs had ascarids, 57 per cent had whipworms, and 71 per cent had hookworms.

Wharton (1917) found 97.45 per cent of the 118 dogs examined in the Philippine Islands infested with intestinal parasites. Only 6.77 per cent of the dogs were infested with *T. limbata*, which is surprisingly low. Wharton states in regard to their small numbers: "The percentage of infestations with this form was much lower than I had expected to find it, and the number of worms present in each case was very small. The fact that the majority of the dogs examined were full grown may account for the small per cent shown. A veterinary surgeon informs me that this parasite is very frequently found in puppies here in Manila, while they are only rarely encountered in older dogs. Ninety-six per cent of his dogs were infested with hook-worm, while there is no record of any whipworm infestation.

A comparison of the figures for 271 infested Detroit dogs, with 76 (Hall) and 48 (Sommer) infested Washington dogs and with 115 (Wharton) infested Philippine dogs shows that worm infestations are more numerous, as might be expected, in warmer climates. Hall finds a greater percentage (57 per cent) of the dogs at Washington, while Sommer finds a lower percentage (28 per cent) of the dogs at Washington to be infested with ascarids than those at Detroit (53 per cent). The percentage of ascarid infestation of both Detroit and Washington

dogs is much higher than Wharton figures (6.77 per cent) for Philippine infestation, which is rather surprising. In regard to hookworm infestation, the figures show just what would be expected. Philippine dogs show the highest percentage of hookworm infestations, 96.6 per cent; Washington dogs rank second, with 71 per cent (Hall) and 56 per cent (Sommer), while Detroit dogs show the smallest percentage, 35 per cent; the farther North we go, the smaller the degree of infestation. In regard to whipworm infestation, we find that Washington dogs have a higher percentage of infestation (57 per cent, according to Hall's figures, and 70 per cent, according to Sommer's) than Detroit dogs (41 per cent).

It thus appears that freezing temperatures of several days' duration would tend to diminish the degree of parasitic infestation, and it therefore seems feasible that manure or feces might be disinfected against most parasitic ova, especially hookworm ova, by being kept at very low temperatures for several days, without destroying the value of the manure as fertilizer, were this procedure practicable.

LITERATURE.

- Hall, Maurice C., 1917. Parasites of the Dog in Michigan. *J. A. V. M. L.*, June, N.S., V. 4 (3), pp. 383-396.
- Sommer, H. A., 1896. Results of an Examination of Fifty Dogs at Washington, D. C., for animal parasites. *Vet. Mag., Phila.*, V. 3 (8), Aug., pp. 48-487.
- Stiles, C. W., 1903. Report upon the Prevalence and Geographic Distribution of Hookworm Disease in the United States. *Bull. Hyg. Lab.* 10, second edition, pp. 1-122.
- Wharton, L. C., 1917. The Intestinal Worms of Dogs in the Philippine Islands. *Ann. Parasit.*, V. 4 (2), pp. 80-82.

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VACCINE THERAPEUTICS.

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Adequate conception of what may be accomplished from the use of vaccines and a recognition of the limitations of this group of products presupposes an understanding of the principles upon which such immunization is based.

It is not my purpose to indulge in a wearisome review of the subject of immunity. There are, however, certain phases involved in the reaction of the body tissues to antigenic treatment, an understanding of which appears essential to a proper perspective of what may reasonably be anticipated from the use of vaccines.

Clinical experience of recent years has proved that the results from immunizing treatment are not entirely attributable to the specific relationship between the antigen injected and the disease treated. A number of infectious conditions—including typhoid fever and certain types of arthritis—have been successfully treated, not only with the vaccines representing organisms unrelated to the infectious process, but with proteins of other than bacterial origin. Various albumoses and proteoses, and even milk, have been thus employed.

The injection of such proteins, especially when made intravenously, is followed by a thermal reaction and by certain other phenomena, including leucocytosis. Such manifestations had previously been noted in connection with intravenous injections of bacterial proteins and were attributed to specific factors. In the light of our present knowledge this classical symptom-complex following the intravenous injections of immunizing agents can not be regarded as entirely of specific origin. Furthermore, any impartial student of immunology must recognize that the non-specific protein reaction is a factor in the therapeutic results of active immunizing treatment, although its importance is secondary

to the specific anti-body production elicited by the administration of such antigens.

The reaction of the body tissues to vaccine treatment may be considered as involving three phases. The first of these is the non-specific protein stimulation to which reference has already been made. This is common to all protein injections.

The second factor is that of protein cleavage or bacterial cell-digestion, a breaking-up in the tissues of the injected micro-organisms. The introduction into the animal body of any foreign protein results in the formation in the tissues of specific "ferments" or "lysins," having for their function the splitting up of that particular type of bacterial protein, and no other. In other words, if egg albumin be injected, the production of ferments specific for the cleavage of this type of protein is brought about by the body cells. If, on the other hand, typhoid bacilli be the injected material, lysins specific for this organism are produced. There is correlated with the production of these lysins, in most cases, the elaboration of other anti-bodies, such as agglutinins and complement-binding substances. The formation of these anti-bodies is to be regarded for the most part as a phenomenon related to cell digestion, although their elaboration is also stimulated to a certain extent by the injection of disintegrated bacteria (endotoxins).

The phase of the immunizing reaction which is probably the most important in the treatment of acute infections is the reaction of the body cells to the end products of bacterial cell digestion; the response to those substances released by the splitting up of the bacteria in the tissues. Correlated with this phase of the immunizing reaction is the production of opsonins.

This conception of the immunizing response of the tissues to antigenic treatment explains why bacterial vaccines have found their chief usefulness either in prophylaxis or the treatment of chronic and subacute diseases; also, why autolysates or bacterial split products have been more successful in dealing with acute fulminating infectious processes. In utilizing bacterial vaccines as prophylactic agents we are endeavoring to build up a comprehensive type of immunity, to equip the tissues with the specific resisting agencies against the particular type of infection involved. The individual who has been immunized with typhoid vaccine

has in his body those anti-bacterial substances whose function it is to destroy the typhoid bacillus. In the treatment of chronic and subacute conditions, and in protracted infectious diseases, such as whooping-cough, the same condition for the most part exists, with the exception that instead of providing against a future foe the body cells are stimulated to mobilize their anti-bacterial activity against one already on the field. On the other hand, in acute infectious processes like pneumonia, the contest between the bacterial vaccine and the pathologic process is too unequal. The force of the attack is so tremendous that the slow mobilization of defensive elements through vaccine administration can be of only limited assistance. The battle is won or lost by the natural resisting forces of the patient before the results of vaccine immunization can exert any profound influence.

It has been previously stated that the therapeutic application of bacterial vaccines finds its chief field of usefulness in chronic and subacute types of infection. Skin diseases, the less acute infections of the respiratory and genito-urinary tracts, and the more protracted infectious diseases, are amenable to vaccine therapeutics. The skin diseases in which vaccines have proved especially useful are furunculosis, sycosis and acne.

Perhaps in no other conditions have better results been obtained than in furunculosis. Disturbed carbohydrate metabolism is, however, an important factor to be considered in these cases. The possibility of diabetes should always be borne in mind, and routine examination of the urine carried out. Aside from diabetes, there is no question but that most cases of furunculosis do better on a restricted carbohydrate diet.

The vaccine treatment of acne is of value only if conducted with persistence. No permanent results are to be anticipated from a few weeks' treatment, although there may be a temporary disappearance of the lesions. Enduring results can be obtained only by immunization extending over a period of months. Furthermore, in many cases of acne of long standing there are malformations of the skin ducts. Vaccine treatment can not correct these anatomical defects, and all that we can reasonably expect under such conditions is a decrease in the inflammation and pustular characteristics of the lesions.

Reports in the literature of successful applications of vaccines

in psoriasis have stimulated considerable interest, but are to be accepted with reservation. There is no specific basis for such treatment, and similar results have been obtained from the use of other protein material, especially when such treatment was combined with the local application of chrysarobin. The results are presumably to be attributed to the protein reaction and not to any specific action of the vaccine.

The value of vaccine treatment in secondary gonorrheal conditions, such as infections of the adnexa and gonorrheal rheumatism, and the uselessness of vaccine therapy in acute urethritis, have been definitely established.

Vaccine therapy has been employed quite extensively and on the whole with favorable results in respiratory infections, including such conditions as acute "colds," recurring infections of the upper respiratory tract and bronchi, and the secondary invasions of asthma and hay fever.

The early use of a mixed vaccine in acute "colds" is undoubtedly of considerable value in increasing resistance and will often abort a "cold." No immediate effects can be anticipated from the use of vaccines after the respiratory passages have become badly congested, although such treatment is probably of some value in shortening the duration of the infection.

The persistent use of vaccines in individuals subject to repeated "colds" is often attended with very satisfactory results. It must be remembered, however, that definite effects can be anticipated only when the susceptibility in such conditions is due to low resistance. Patients harboring infective foci, such as diseased tonsils and alveolar pus pockets, can not be expected to respond to vaccine treatment until these foci are removed.

Vaccine treatment can not be expected to cure asthma or hay fever, but may be of considerable value in these diseases.

The value of vaccines in asthma is presumably dependent upon two factors: First, the stimulating effects of the protein content, and, second, the relief of bacterial irritation in the bronchi. Undoubtedly the irritation of the bacterial invaders is a frequent cause of asthmatic paroxysms.

In hay fever bacterial infection is a secondary factor, but one which may contribute very heavily to the distressing symptoms of the disease. Immunization directed against the relief of this

infectious element is often of considerable value, but should be combined when possible with the use of the specific pollen concerned.

During the last few years considerable use has been made of vaccine therapy in ozena, and the results on the whole appear to have been distinctly favorable. The same is true of vaccine treatment in otitis media.

Variable reports have been made concerning the results obtained from the use of vaccines in such acute infections as whooping-cough, scarlet fever, erysipelas, typhoid, pneumonia and puerperal sepsis.

There is no question, in the writer's judgment, as to the value of pertussis vaccines when used in sufficient dosage in the treatment of whooping-cough. The duration of the disease is usually shortened, there is less tendency to paroxysms and vomiting, also fewer complications.

There seems reason to believe also that in erysipelas, scarlet fever and typhoid, vaccines are of some value. The value of vaccines in pneumonia and severe types of puerperal sepsis is negligible, as their action is too slow to cope with the violence of the pathologic process. The hope of successful immunization in such cases, and the best results in the treatment of such conditions as erysipelas and typhoid, lie in the utilization of autolysates or bacterial split products. Serum treatment is useful in pneumonia due to a certain type of pneumococcus, the so-called Type I.

It is desired to refer briefly in conclusion to the frequently raised question of the respective values of autogenous and stock vaccines.

Turning over the leaves of medical publications, we not infrequently see the title "Autogenous Versus Stock Vaccine," as though these were diametrically opposed and irreconcilable lines of medication. There is nothing about autogenous and stock vaccines which should bring them in conflict, nor would this spirit of controversy arise so frequently if the discussions were not often tinged with personal interests. Both lines of treatment are of value and each has its definite indications.

Theoretically, autogenous vaccines are ideal, and there is no question but that in their practical application brilliant results are often accomplished. There are, however, certain disadvantages

which militate against the universal use of autogenous preparations.

In the first place, technical difficulties exist. An autogenous vaccine, to be of the greatest efficiency, must be made by a well-trained bacteriologist with ample facilities for carrying out the necessary work. Such conditions do not universally prevail. The sending of the specimen by the clinician to a considerable distance is not always practical, because the physician may not understand how the culture should be taken or the proper media to employ, and because many of the more delicate pathogenic bacteria, such as the gonococcus, will not survive more than twenty-four hours on recent isolation, and are killed by vicissitudes in temperature in a much shorter period of time. Again, such organisms as the acne bacillus can be isolated only by a laboratory man with unusual experience, and then often after weeks or even months of cultural work.

The question as to whether an autogenous vaccine is prepared with a view of affording the highest immunizing value or thrown together with the least expenditure of time, expense or effort, depends, of course, upon the honesty and competency of the laboratory undertaking its preparation. Not infrequently in their preparation no attempt is made to isolate the various bacteria contained, the growth being taken from the original culture, killed and standardized. It is a fact generally recognized in bacteriologic work that non-pathogenic bacteria commonly grow luxuriantly under artificial conditions, whereas the more virulent disease-producing bacteria grow reluctantly under the same circumstances; consequently it is obvious that a vaccine prepared as just described would in many cases represent a preponderance of contaminating non-essential bacteria and a few or none of the organisms actually concerned in the infection. Aside entirely from these technical questions, it must be recognized that autogenous strains of bacteria do not always constitute good antigens. There often exists in chronic types of infection a condition of "symbiosis," or mutual tolerance, between the invading bacteria and the immunizing forces of the patient. The use of autogenous strains under such conditions may be quite valueless. This has been demonstrated both by laboratory work on the animals and clinical observation of the opsonic index in chronic infections.

The use, in such patients, of polyvalent stock vaccines, representing strains selected for their high antigenic value, constitutes a much more promising therapeutic measure.

The conditions in which autogenous vaccines would be of the greatest efficiency are the acute infections, since here we are dealing with active strains of bacteria. Unfortunately, however, in most of these the time element renders their use impracticable, since the physician can not wait for from three days to a week to have a vaccine prepared.

Finally, vaccine therapy is by no means to be regarded as a panacea, but, intelligently applied, constitutes a valuable adjunct to the other measures available for dealing with various types of infection.

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THE EFFECT OF ALCOHOL ON PITUITARY EXTRACT.

BY HERBERT C. HAMILTON.

From the Research Laboratory of Parke, Davis & Co., Detroit, Michigan.

It not infrequently happens that when a salesman, detail man or other member of a firm of manufacturers of pharmaceutical products is confronted with the statement that a certain preparation fails to act in its accustomed manner, he points out a number of possible factors in attempting to find the cause in the particular case.

Pittenger presented a paper before the Scientific Section of the A. Ph. A. 1918 meeting (published in the October issue of the *Journal*) in which he notes one such instance where the well-known fact that alcohol precipitates the active constituent of pituitary extracts had been advanced as the possible explanation of a failure of this preparation to act on the uterus muscle.

The writer, whose discussion of this paper on the floor is not given in the published proceedings, corroborated the facts there presented and noted that the question had come up on more than one occasion and that laboratory experiments had in every case shown that alcohol in the quantity present could not affect the activity of this preparation unfavorably.

The subject seems important enough to be carried somewhat further in order to explain fully the conditions under which alcohol can affect the pituitary extract unfavorably.

The writer has observed that a commercial sample of pituitrin shows an opalescence from the action of strong alcohol but that a mixture of equal parts pituitrin and 95% alcohol shows no permanent opalescence and no precipitate. Diluted and injected into the circulation of an anesthetized dog in the usual method of testing, there is no perceptible lowering of its activity. This is

very much in excess of the possible alcohol content from washing the syringe or the site of injection.

A further experiment has been carried out on a highly active dry pituitary product. This material was ground in a mortar and rubbed thoroughly with 95% alcohol, adding successive portions and filtering the alcohol to obtain a clear solution.

Three series of tests were made on the resulting products, namely, tests of the dry material after being washed with alcohol, tests of the residue remaining on recovery of the alcohol, first an aqueous solution of this residue and second a hydro-alcoholic solution of the residue.

The results of these tests showed that 95% alcohol is not a solvent for the active principle nor has it any deleterious action; the active agent was no less active, and the residue from evaporation of the alcohol had neither pressor nor oxytocic activity.

The only reaction between alcohol and pituitary extracts is when the former is present in great excess, in which case it acts as a precipitant.

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**STUDIES ON DERIVATIVES OF TRIHALOGEN
TERTIARY-BUTYL ALCOHOLS.**

**II. The Propionic and Butyric Esters of Tribromotertiary-butyl alcohol
(Brometone).**

BY T. B. ALDRICH.

From the Research Laboratory of Parke, Davis & Co., Detroit, Michigan.

A continuation of my studies on derivatives of esters of trihalogen tertiary-butyl alcohols has led to the preparation of the propionic ester as well as the butyric ester of brometone. In several attempts to prepare these esters direct from the acids, using zinc chloride as suggested by R. Wolffenstein, A. Loewey and M. Bachstetz¹ in the preparation of chloretone esters, decomposition apparently took place without formation of the ester, invariably giving rise to a volatile substance very irritating to the eyes. This method was therefore discarded and the ester prepared by allowing the acid chloride to act on the alcohol. As has been emphasized in one of my previous articles² on the subject of esters, it is desirable to dehydrate the halogen alcohol as much as practicable by allowing it to stand in a desiccator over calcium chloride (not sulfuric acid), thus economizing material.

The propionyl chloride, as well as the butyryl chloride, was prepared in the usual way by allowing phosphorus trichloride to act on the purified acid. The fraction of propionyl chloride boiling at 76 to 77.5° and that of butyryl chloride boiling at 99 to 100.5° (uncorr.) were employed in the preparation of the respective esters.

¹*Ber.*, 48, 2035 (1915).

²T. B. Aldrich and C. P. Beckwith, *This Journal*, 38, 2740 (1916).

PROPIONIC ESTER OF BROMETONE.

Four parts of brometone were placed in a suitable flask and one part of the propionyl chloride added. Most of the brometone immediately dissolved, and hydrochloric acid gas was given off. The last portions of the gas were driven off by heating for some time on the steam bath. The contents of the flask were then shaken with 100 Cc. of 10% sodium hydroxide, the mixture heated on the steam bath for a short time, thoroughly washed out with water and extracted with ether.³ The yield in an impure form in one instance was 73% of the theoretical. Recrystallized from hot alcohol, white crystals melting at 27° were obtained.

Bromine determinations (Carius) carried out with a product recrystallized several times from moderately strong alcohol gave the following results:

Subst., 0.2276, 0.1987, 0.2237 g. Bromine, 0.1492, 0.1288, 0.1466 g.
Calc. for $C_3H_5O_2Br_2$: Br, 65.39. Found: 65.55, 64.79, 65.53.

These results are sufficiently near the theoretical requirement to characterize the compound as the propionic ester without the necessity of a combustion analysis.

The ester is extremely soluble in strong alcohol, acetone, chloroform, ether, glacial acetic acid, benzene, etc., practically insoluble in water. It is precipitated as an oil from its alcoholic solution by water. The odor resembles that of the acetic esters very closely. Boiling with water or 10% sulfuric acid decomposes the ester slowly, while boiling with 10% caustic soda decomposes it more rapidly. It is decomposed into the alcohol and acid very quickly by boiling a few minutes with an excess of conc. nitric acid, conducting itself in this respect toward hot nitric acid in the same way as do the acetic esters, since it is necessary only to cool after boiling and add water when the alcohol is thrown out in the form of a white solid. The compound volatilizes very slowly in the air, and is characterized, similar to the other ester thus far studied, by its general stability.

R. Wollfenstein, A. Loewry and M. Bachstetz¹ state briefly

³On adding *very cold* water during the process of washing, the oil solidified completely; on warming to body temperature, however, it melted.

¹*Ann.*, 49, 206 (1903).

that the *propionic acid ester of chlorotone* is a yellowish oil boiling at 88 to 90°, at 14 mm. It has not been determined exactly where the brometone ester boils, but it has been shown with certainty that it boils higher than does the chlorotone ester.

BUTYRIC ESTER OF BROMETONE.

In the preparation of this ester, as previously mentioned, butyryl chloride boiling at 99 to 100.5° was employed.

The preparation of the ester as well as its purification was carried out in general according to the method already described under the propionic ester.

After drying the ethereal extract over calcium chloride the ester was fractionated under reduced pressure and a fraction boiling fairly constant at 144 to 145° under 13 to 14 mm. pressure obtained.

Bromine determinations (Carius) carried out with this fraction gave the following results:

Subst., 0.2137, 0.2035 g. Bromine, 0.1351, 0.1291 g.

Calc. for $C_4H_7O_2Br$: Br, 63.00. Found: 63.19, 63.44; average, 63.21.

This ester has approximately the same solubilities as the propionic ester and its odor resembles that of the other aliphatic ester in general, but there is a faint suggestion of the presence of butyric acid. It conducts itself toward boiling water, sulfuric acid, alkali or conc. nitric acid the same as the propionic ester.

The compound is slightly volatile with steam; in the air it volatilized very slowly. It is apparently less volatile than either the acetic or propionic ester.

The following preliminary data relative to the pharmacological tests were kindly furnished me by my associate, Mr. L. W. Rowe:

"Neither the butyric nor the propionic ester of brometone appears to possess anesthetic properties. The propionic ester is the more readily absorbed following hyodermic injection and is much less irritating than the butyric ester. Neither ester exerts a very appreciable action upon the heart or general circulation, as the intravenous injection of a rather large dose of each into an

anesthetized dog caused only a very slight fall in blood pressure. They are both comparatively inactive pharmacologically, due to the probable fact that they are not decomposed into soluble constituents having a typical physiological action and are rather slowly absorbed. In this respect they are similar to other esters of this series."

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LUNGWORM DISEASE IN A TEN-YEAR-OLD HORSE.

H. PRESTON HOSKINS, V.M.D.

From the Research Laboratory, Parke, Davis & Company, Detroit, Michigan.

In certain years, and in quite widely separated localities, losses caused by lungworms in pigs, lambs and calves are very heavy, and result in serious financial losses to the owners of the animals. Curative measures are eminently unsatisfactory in most cases. Verminous bronchitis, verminous pneumonia, and verminous broncho-pneumonia, names given to the pathological condition produced by the presence of the worms, is almost uniformly regarded as a condition which affects young animals only. Therefore the case here reported, in a ten-year-old horse, may be of interest.

In speaking of the occurrence of the disease, Hutyra and Marek state:

"Among domestic animals, sheep and goats are most commonly affected, in some neighborhoods also swine; more rarely affected are cattle and camels; horses, asses, cats, dogs, and rabbits only exceptionally."

From this statement it would appear that lungworm disease in the equine species is comparatively rare. Under anatomical changes, the same authors state:

"In horses and asses the disease occurs exceptionally and only leads to a clinical picture similar to that seen in verminous bronchitis of calves. Fatal cases have been observed repeatedly in asses (Stewart)."

The usual explanation given for the predominance of the disease among young animals, as compared with mature stock, is the lessened resistance offered by the young animals, a reason which holds for most all diseases, infectious, constitutional, and parasitic as well. A point that should not be lost sight of, in this

connection, is that an established lungworm infestation in a young animal would undoubtedly cause a more profound disturbance than the same infestation in a mature animal of the same species. Verminous infestations of the lungs may be more common among old animals than we have been led to believe, in fact it is not uncommon to meet this condition post-mortem in apparently healthy animals, especially hogs and sheep, examined at large slaughter houses.

The present case was a bay gelding (No. 412), weighing about 1250 pounds, ten years old, and purchased in July, 1915, at St. Louis, Mo., brought to Parkedale Farm, Rochester, Mich., and used for the production of antidipltheric serum. This horse did not prove to be a profitable yielder of potent serum, so was transferred to the thyroidectom list in April, 1916, after having been thyroidectomized.

During 1917 this horse was somewhat unthrifty and it was believed that this condition was a sequel to the removal of the thyroid glands, although most horses so treated do not begin to show symptoms so soon following the operation. A rather characteristic chain of symptoms is frequently noted in horses that have been subjected to thyroidectomy. In this horse, after viewing the autopsy, it was believed that the combination of athyroidism and verminous pneumonia contributed to an earlier cachexia than either of the conditions might have produced independently.

The condition of the horse became hopeless and he was destroyed November 5, 1917, and a careful autopsy held. On exposing the thoracic viscera, the writer was struck by the appearance of the lungs. Having seen numerous cases of verminous broncho-pneumonia in pigs, and a number of cases in lambs and calves, the pathological picture presented by the lungs was pretty well impressed upon my memory, and the condition was immediately suspected on seeing the lungs of the horse in question.

The basal borders of both lungs were quite extensively affected with a chronic interstitial pneumonia, which was probably the result of a chronic bronchitis. The lung tissue was pale, a grayish yellow in color. The texture was tough and the consistency firm, due to the formation of new connective tissue,

especially in the interlobular spaces, where it appeared quite prominently. Some edema was in evidence, and the affected portions of the lungs were atelectatic. On section the bronchi and larger bronchioles showed the presence of large numbers of thread-like worms. These were matted together with a considerable amount of mucous exudate.

The worms were identified by Mr. Meyer Widgor, of this laboratory, as *Dictyocaulus micrurus*. According to Hutyra and Marek this variety occurs in cattle, fallow-deer and stags; exceptionally in horses and asses. All organs, with the exception of the lungs, as above noted, appeared to be normal.

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Vol. 63, No. 2.)

BOTANICAL NOMENCLATURE OF THE U. S. P. IX.

**A Survey of the Botanical Nomenclature of the New Pharmacopœia
Discloses the Fact that Neither the Vienna nor the American Code
was Strictly Adhered to by the Nomenclatorial Committee.**

BY OLIVER ATKINS FARWELL.

A careful examination of the botanical nomenclature of the new revision of the Pharmacopœia discloses the fact that the authors did not invariably follow either the "Vienna" code or the "American," but either one or the other as it suited their convenience, and in some instances neither. In most instances where forms of a species, other than the type, are admitted, the trinomial is used; as *Glycyrrhiza glabra glandulifera*; in many cases, however, the "variety" is used as in *Melaleuca Leucadendron* var. *Cajeputi*. The former typifies the American code, which does not recognize the rank of *variety*—the trinomial being the method of designating a *sub-species*; the latter is characteristic of the Vienna system of nomenclature. The system of considering a variation of a species as a subspecies and designating it by a trinomial (the American Code) should be discontinued, as an application of the rule simply makes authors, who do not follow the code and the older authors of a bygone day, express a classification which they had no intention or thought of conveying.

Apparently the American code has been the guiding star of the nomenclatorial committee, but it has balked when a strict application of the rules would have produced a repeating binomial, one where the generic and specific names are the same, as *Zingiber officinale* for *Zingiber Zingiber*. Geographical specific names are decapitalized, a feature that is greatly to be deprecated. Such names are proper names in just the same manner as are specific names derived from old generic names or

from the names of persons and they should not be treated differently. Just so long as English type is used to express a binomial, just so long should the rules governing English grammar and syntax be followed. If decapitalization is desired, the binomial should be expressed in Roman type, *i.e.*, in small capitals. There are a good many exceptions to the rule that the name of a family of plants should end in "aceæ," as *Gramineæ*, *Leguminosæ*. In each instance the ending should be changed to "aceæ" so as not to conflict with the nomenclature of other botanical categories.

The following notes and suggestions may be of service in the preparation of future editions.

AGAR. This article is said to be the dried mucilaginous substance obtained from the *Gracilaria lichenoides* Greville and other algæ of the sea coast of Asia, especially from species of *Gelidium* and of *Gloiopeltis*. It is generally conceded that the agar derived from *Gracilaria lichenoides* is the dried, *unaltered* thallus, and is known to the pharmaceutical and commercial worlds as Ceylon agar. Some species of *Gloiopeltis* yield a glue while others are used as a food. Japanese agar is derived from *Gelidium corneum* (Hudson) Lamour, *G. cartilagineum* Gaillon and perhaps from other species of *Gelidium*. Japanese agar is a gelatinous substance, *gelose*, extracted from the algæ. The commercial agar brought to this country for medicinal purposes comes from Japan and is not an unaltered thallus but an extracted gelatinous substance, and therefore corresponds to the article known as Japanese agar as above described. The definition should be corrected to exclude species of *Gracilaria* and *Gloiopeltis* as sources of origin of agar. The writer of this paper can see no good reason for substituting a class name for this alga instead of the family name. "Fam. Gelidiaceæ" should be used instead of "Class Rhodophyceæ."

AMYGDALA DULCIS, OLEUM AMYGDALÆ AMARÆ, OLEUM AMYGDALÆ EXPRESSUM. The sweet almond is said to be derived from *Prunus Amygdalus dulcis* De Candolle and the bitter almond from *Prunus Amygdalus amara* De Candolle. De Candolle is not the author of the above combinations. He did not name them under *Prunus*, but under *Amygdalus* and as varieties, not as subspecies; the citation of De Candolle as the author of the combinations is, therefore, without authority. The better

way is to keep *Amygdalus* separate from *Prunus*. The bitter almond would then be derived from *Amygdalus communis* Linne, and the Sweet from *Amygdalus communis* Linné var. *dulcis* (Miller) De Condolle. It is not necessary to use the variety *amara* for the bitter almond, as it is but a synonym of the species. However, if they are to be retained under *Prunus*, *P. Amygdalus* Stokes is not the proper name for the species under any code of nomenclature now followed, all of which recognize the law of priority. Hudson, in 1778, published a *Prunus communis* to include *P. domestica* Lin., *P. spinosa* Lin., and *P. insititia*, Lin., all of which antedate the species of Hudson; consequently Hudson's *P. communis* is but a synonym that can never be reinstated and therefore can not bar the legitimate use of the name for another species. According to the laws of priority the proper designation of the almonds under *Prunus* is herewith given.

PRUNUS COMMUNIS (Lin.) Farwell, nov. comb.

Amygdalus communis Lin. Sp. Pl. 473, 1753.

Amygdalus communis Lin. var. *amara*, D. C. Fl. Fr. IV 486, 1805 and Prod. II, 530, 1825.

PRUNUS COMMUNIS (Lin.) Farwell, var. *DULCIS* (MILL.) Farwell, nov. comb.

Amygdalus dulcis Miller Dict. Ed. 8 No. 2, 1768.

Amygdalus communis Lin. var. *dulcis* D. C. II. cc.

ASPIDIUM. The oldest post-Linnæan generic name for the male fern is *FILIX* (Fuchs) Hill. The proper combinations for the species designated are *Filix Filix-mas* (Lin.) Farwell and *Filix marginalis* (Lin.) Farwell.

ASPIDOSPERMA. The specific name *quebracho blanco* is written as two words, the hyphen being omitted; this doubtless is a typographical error; nevertheless, as written, it becomes a trinomial and, under the American Code, indicates that the drug is derived from a subspecies *blanco* of the species *Aspidosperma Quebracho*.

AURANTII DULCIS CORTEX, OLEUM AURANTII. The peel and oil of the sweet orange are said to be derived from the *Citrus Aurantium Sinensis* Galesio. Just why this name should be attributed to Galesio is a mystery; Linnæus (Sp. Pl. 783, 1753) was the first to use it and he should be quoted as the author. It might be better to consider this as a distinct species under the name *Citrus Sinensis* (Lin.) Osbeck.

AURANTII AMARÆ CORTEX. The bitter orange peel is said to be derived from *Citrus Aurantium amara* Lin. Why any varietal or subspecific name should be used is a question that has not been explained. The bitter orange (*Citrus vulgaris* Risso, *Citrus Bigaradia* Loisel, and *Citrus Aurantium amara*) is the exact type of the Linnæan *Citrus Aurantium*. No further designation is necessary.

CANNABIS. Cannabis is said to be derived from *Cannabis sativa* Linné or its variety *Indica* Lamarck. We have American, Mexican, African, Indian, etc., cannabis; but these are geographical or commercial terms to designate the country of origin. Why it should be necessary does not appear, as the species from one country, when properly prepared, is as active as from another. So we have the pharmaceutical term cannabis sativa variety *Indica* (not botanical) to designate the Indian grown drug. To quote Lamarck as the author of a botanical variety *Indica* is absurd; there has never been, in so far as I have been able to ascertain, a properly described botanical variety under the name of *Indica*. Lamarck described a species, *Cannabis Indica*, which was later reduced to synonymy, this form not being given any recognized rank of any degree.

CARDAMOMI SEMEN. The botanical origin is given as *Elettaria Cardamomum* White et Maton. The correct combination and author citation under this genus is *Elettaria Cardamomum* (Lin.) Maton and is based on the *Amomum Cardamomum* Lin. Sp. Pl. 1, 1753. The authors of the Index Kewensis and K. Schumann in Das Pflanzenreich IV, No. 46, p. 238, cite the Linnæan binomial as *Amomum Cardamon* and apply it to the Java cardamom plant. A reference to the Species Plantarum will show that Linnaeus did not use the specific name *Cardamon* but wrote **CARDAMOM**, which is an abbreviation for *Cardamomum* just as *gran. parad.* on the next page (?) is for *Granum-paradisi*. I have not been able to ascertain who was the first author to use the specific name *Cardamon*, but Linnaeus certainly did not use it. The genus **AMOMUM** was founded by Linnaeus in 1736 on the small cardamoms of the shops. The ginger was included but no part of the description was drawn from it. It is therefore very doubtful if the name can rightfully be used for any other plant.

Certain elements of three distinct species entered into the make-up of the Linnæan *Amomum Cardamomum*, but the confusion over these species was not original with Linnæus. His description was taken from his earlier *Flora Zeylanica*, which also is the first reference given after the description in the *Species Plantarum*. A reference to the *Flora Zeylanica* develops the fact that this species, as well as the genus *AMOMUM* as above shown, was founded on the small cardamoms of the shops. The only correct interpretation of the genus *AMOMUM* would be to retain it for the plant on which it was founded, hence the proper name for our cardamoms is *Amomum Cardamomum* Lin. The genus to which Roscoe in 1806 transferred the name *AMOMUM* should probably be known as *MEISTERA*, Giseke (1792).

CARYOPHYLLUS, OLEUM CARYOPHYLLI. The proper authority for "*Eugenia aromatica* (Linné)" is "Baillon" not "O. Kuntze" as given in the *Pharmacopœia*. Baillon made the combination in his *History of Plants*, Vol. VI, pp. 311 and 345, 1877, 14 years ahead of O. Kuntze. But this name is not tenable because of an earlier, valid species of the same name, *Eugenia aromatica* Berg. 1854. The proper name under *EUGENIA* is *Eugenia caryophyllata* Thunb. The synonym "*Jambosa Caryophyllus* (Sprengel) Niedenzu" should be enclosed in marks of parenthesis.

CINNAMOMUM ZEYLANICUM. The proper binomial for this product is *Cinnamomum Cinnamomum* (Linné) Karsten.

ERIODICTYON. The correct authority for "*Eriodictyon Californicum* (Hooker and Arnott)" is "Torrey" not "Greene" as given in the *Pharmacopœia*.

EUCALYPTOL, EUCALYPTUS, OLEUM EUCALYPTI. The specific name "Globulus" should not be capitalized; it is not a proper name.

FÆNICULUM, OLEUM FÆNICULI. The correct name for the source of these drugs is *Feniculum Fœniculum* (Linné) Karsten.

GELSEMIUM. The proper authority for the binomial "*Gelsemium sempervirens* (Linné)" is "Persoon" not "Aiton filius," the former having made the combination in 1805, six years ahead of the latter.

GLYCYRRHIZA. The designation *Glycyrrhiza glabra* Linné is sufficient to indicate the source for Spanish licorice. The custom

of making a species an indefinite entity and then giving varietal name to what may be considered the typical form can not be too severely censured. Nothing is to be gained by it. "(Waldstein et Kitaibel)" should be inserted between "*glandulifera*" and "Regal et Herder" in order to make the author citation perfect.

IPECACUANHA. The source of ipecac is given as *Cephaelis Ipecacuanha* (Brotero) A. Richard and *Cephaelis acuminata* Karsten. The oldest generic name for the ipecacs is *Ouragoga*, published by Linnæus in 1737 in the first edition of the *Genera Plantarum*, 378, and in Hort. Cliff., 486. Also as a post-Linnæan name in December, 1774, in a dissertation on *Viola Ipecacuanha* by Daniele Wickman, later appearing in Schreber's edition of the *Amoenitates Academicæ* in 1785, Vol. VIII, 240, 241, 243. In the index of the first edition of the *Genera Plantarum* the name was listed as *Uragoga* and in this form was adopted by Baillon and later by O. Kuntze to include not only the ipecacs (*Cephaelis*) but also a number of closely allied genera (*Psychotria*, *Callicocca*, *Mapouria*, etc.). K. Schumann, in Engler and Prantl's *Pflanzenfamilien*, used the name for the genus *Cephaelis* alone, restoring to generic rank those genera that had been reduced by Baillon and by Kuntze. "*Uragoga*," as spelled by these authors, is not a valid post-Linnæan name. *Ereca* Aublet 1775 has been taken up recently by Standley for *Cephaelis*, but this is later by a fraction of a year than *Ouragoga* and therefore is not tenable. *Uragoga acuminata* (Bentham) OK. is a species of *Psychotria* and does not apply to the Carthagena ipecac. The proper combinations to designate the ipecacs are as herewith given.

OURAGOGA IPECACUANHA (Brotero) Farwell, nov. comb.

Callicocca Ipecacuanha Brot. Trans. Linn. Soc. VI, 137, pl. 11, 1802.

OURAGOGA ACUMINATA (Karsten) Farwell, nov. comb.

Cephaelis acuminata Karsten, Deutsche Flora p. 1196, 1880-1883.

LIMONIS CORTEX, OLEUM LIMONIS. The botanical source of the lemon is *Citrus Medica* Lin. var. *Limon* Lin. This is the oldest name and should be adopted in preference to the later one of Hooker filius; *Citrus Limonia* Osbeck, if as a distinct species.

MALTUM. The botanical source is given as *Hordeum sativum* Jessen. This is but a synonym and should give way to the valid name, *Hordeum vulgare* Lin.

MENTHA VIRIDIS, OLEUM MENTHÆ VIRIDIS. The botanical origin of this drug is said to be *Mentha spicata* Lin. (*M. viridis* Lin.). There seems to be little or no excuse for making *M. viridis* Lin. a synonym of *M. spicata* Lin. or attributing the source of garden spearmint to the latter species. In the *Species Plantarum*, ed. I, Linnaeus had *M. spicata* with three named varieties, *viridis*, *longifolia*, and *rotundifolia*. In the second ed., *M. spicata* with the variety *longifolia* becomes *M. sylvestris*, and the varieties *viridis* and *rotundifolia* are elevated to specific rank under their respective names. *M. spicata* Lin. is, therefore, the older and valid name for the plant that has been more commonly known as *M. sylvestris*, and the spearmint of cultivation and of pharmacy is *M. viridis*. *M. spicata* should be dropped.

MYRRHA. Myrrh is said to come from one or more species of *Commiphora*. The oldest name and consequently the valid one is **BALSAMEA**. It should be adopted.

OLEUM CAJUPUTI. The botanical source of this oil is said to be *Melaleuca Leucadendron* Linné, especially the variety *Cajuputi* Roxburgh and the var. *minor* Smith. Neither Smith nor Roxburgh are the authors of the varieties mentioned; they published their respective names as specific names. The correct author citation will appear in the synonymy to be given below. The oldest post-Linnaean name for this group of plants is **KAJUPUTI**, Adanson *Fam. Pl.* II, Index, page 530, 1763. On page 84, vol. 2, Adanson has the generic name *Caju puti* as two distinct words, which, of course, is not tenable as a valid generic name; but on page 530 in the Index he has **KAJUPUTI** with a reference to *Rumph. 2 t. 16* and to page 84, where the description is to be found. The proper binomials are as herewith given.

KAJUPUTI LEUCADENDRA (Lin.) Farwell, nov. comb.

Myrtus Leucadendra Lin. *Syst.* ed. 10, 1056, 1759.

KAJUPUTI LEUCADENDRA (Lin.) Farwell variety **ANGUSTIFOLIA** (Lin. fil.) Farwell, nov. comb.

Melaleuca Leucadendra Lin. var. *B. angustifolia* Lin. fil. *Suppl. Pl.* 342, 1781.

KAJUPUTI LEUCADENDRA (Lin.) Farwell variety **MINOR** (Sm.) Farwell, nov. comb.

Melaleuca minor Sm. *Rees, Cycl.* 23, 1797.

Melaleuca Cajuputi Roxburgh *Fl. Ind.* III, 394 1832.

Melaleuca Leucadendron Lin. var. *minor* (Sm.) Duthie in Hk. f Fl. Brit. Ind. II 465, 1778.

Maleleuca Leucadrendron Lin. variety *Cajeputi* (Roxb.) Niedenzu in Engler and Prantl's Pflanzenfamilien III Teil, 7 abt. 95 and 96, 1892.

The species is founded on the *Arbor alba* Rumph. 2, 72, t. 16, and the second variety on the *Arbor alba minor* Rumph. 2, 76, t. 17 fig. 1. Some authors consider the two varieties named above as identical, in which case the first named would be the valid one, as it is the oldest. The second variety is the one that produces the greater part of the commercial cajuput oil.

OLEUM CHENOPODII. The source is given as *Chenopodium ambrosioides anthelminticum* Linné. The author citation* for the variety *anthelminticum* is (Linné) A. Gray. Linnæus is not the author of a subspecies *anthelminticum*.

OLEUM LAVANDULÆ. The valid designation of the lavender plant is *Lavandula Spica* Linné, not *L. vera* DC., which is a later synonym. In any event *L. vera* DC. is not the name to use; the earliest name after that of Linnæus, in case his should be discarded (for which there is no excuse), is *Lavendula angustifolia*, Miller.

OLLUM PIMENTÆ. *Pimenta Pimenta* (Linné) Lyons is the valid binomial for the source of this product; not *P. officinalis* Lindley.

OLEUM SASSAFRAS, SASSAFRAS. *Sassafras Sassafras* (Lin.) Karsten is the proper combination to designate the sassafras.

OLEUM SESAMI. The proper binomial to designate the sesame is *Sesamum orientale* Lin.; not *S. Indicum* Lin.

PETROSELINUM. *Petroselinum hortense* Hoffman has precedence over *Petroselinum sativum* Hoffman but the valid binomial is *Petroselinum Petroselinum* (Linné) Karsten.

SPARTILENÆ SULPHAS. The specific name in *Cytisus scoparius* (Linné) Link should be decapitalized. It is not an old generic name or a vernacular name, just an adjective.

TARAXACUM. The botanical origin is given as *Taraxacum officinale* Weber. The proper designation under taraxacum is *Taraxacum Taraxacum* (Linné) Karsten. But *Taraxacum* is not the oldest generic name and for that reason is not the valid one. *Leontodon* Lin. was founded in 1737 on the common dan-

delion, the *Dens Leonis* of the older botanists. As the genus appeared in the first edition of the *Species Plantarum*, it must be accepted for the species on which it was founded, the dandelion, which is *Leontodon Taraxacum* Linné. The genus generally known as *Leontodon* of late years is *VIREA* Adanson.

ULMUS. The source of origin is given as *Ulmus fulva* Mx. "*Ulmus pubescens* Walter" is generally considered to apply to the same species and being the older name by 15 years should be adopted.

XANTHOXYLUM. The proper spelling for this generic name is *Zanthoxylum*. Linnæus used Z for the initial letter, but Miller changed it to X. The original spelling should be restored.

ZINGIBER. The source of origin is given as *Zingiber officinale* Roscoe. The proper appellation is *Zingiber Zingiber* (Linné) Karsten.

In order to bring about a uniformity in family nomenclature, each name ending in "aceæ" and the oldest family name being used, the following changes are necessary:

Gramineæ	to	Graminaceæ.
Palmæ	to	Palmaceæ.
Fagaceæ	to	Castaneaceæ.
Moraceæ	to	Lupulaceæ.
Polygonaceæ	to	Persicariaceæ.
Chenopodiaceæ	to	Blitaceæ.
Cruciferae	to	Cruciferaceæ.
Leguminosæ	to	Leguminaceæ.
Euphorbiaceæ	to	Tithymalaceæ.
Rhamnaceæ	to	Zizyphaceæ.
Sterculiaceæ	to	Cacaoaceæ.
Ternstroemiaceæ	to	Camelliaceæ.
Guttiferæ	to	Hypericaceæ.
Punicaceæ	to	Granataceæ.
Umbelliferæ	to	Umbellataceæ.
Oleaceæ	to	Jasminaceæ.
Loganiaceæ	to	Strychnaceæ.
Hydrophyllaceæ	to	Hydroleaceæ.
Labiatae	to	Labiataceæ.
Rubiaceæ	to	Aparinaceæ.
Cucurbitaceæ	to	Bryoniaceæ.
Compositæ	to	Compositaceæ.

BOTANICAL NOMENCLATURE OF THE PHARMACOPOEIA.

In Which the Author Supplements One of His Papers Appearing in the
Circular for April of Last Year—Similar Articles of His,
Published or Yet to Appear, Are on the Nomenclature
of the National Formulary.

BY OLIVER ATKINS FARWELL.

ALOE. Under this title the United States Pharmacopœia gives *Aloe vera* LINNÉ as the source of Curaçao Aloes. There is no doubt but that this species produces the true Barbadoes Aloes; there is still some doubt as to whether the Curaçao product is derived from the same form. Planters claim that the Barbadoes and the Curaçao products are identical; nevertheless, Holmes, who has given the subject an exhaustive study, insists that they are distinct, giving the *Aloe Chinensis* BAKER as the source of the Curaçao. All things considered, it seems best to adopt an intermediate course and give the source of Curaçao Aloes as *Aloe vera* LINNÉ, variety *Chinensis* (J. A. and J. H. Schultes) Berger. Berger in *Das Pflanzenreich*, Heft 33 p. 230, quotes Haworth as the author of the above combination, but that is a manifest error. Haworth described the plant as a variety b. of *A. Barbadosensis*, but did not give it a name.

BENZOENUM. *Styrax Benzoin* DRYANDER is given as the source of Sumatra Benzoin, but no more definite source for the Siam is given other than "some other species of *Styrax*." Holmes in the *Pharmaceutical Journal* for November 29, 1913, gives as the source of the Siamese Benzoin, *Styrax Tonkinense* CRAIB; in the same journal for September 8, 1917, the source is said to be the *Styrax Siamense* RORDORF, a recently described species. As both these species come from the heart of the Siam Benzoin district, and both yield a similar resin, the inference to be drawn is that each yields a part of the commercial product. These names, therefore, should be adopted rather than the indefinite "some other species."

ASPIDOSPERMA. The oldest generic name and consequently the proper one is *Macaglia* RICHT. The proper binomial is *Macaglia Quebracho-blanco* (Schlechtendal) LYONS.

BUCHU. *Parapetalifera* Wendland has a year's priority over *Barosma* Willdenow. The species should be as follows:

PARAPETALIFERA BETULINA (Thunberg), n. comb. *Diosma betulina* Thunberg, Prod. Pl. Cap. 43, 1794-1800.

Barosma betulina (Thunberg) Bartling and Wendland filius. Diosm. 102, 1824.

PARAPETLIFERA SERRATIFOLIA (Curtis), n. comb.

Diosma serratifolia, Curtiss, Bot. Mag. t. 466, 1799.

Parapetalifera serrata Wendland, Coll. Bot. 92 t. 34, 1808.

Another species, intermediate between the two, but not now official, and one that has often been collected and mixed in with the former species, is:

PARAPETALIFERA CRENULATA (Linné), n. comb.

Diosma crenulata Linné, Cent. Pl. 2, 11, 1756.

Parapetalifera odorata Wendland, Coll. Bot. 1, 50, t. 15, 1808.

Parapetalifera is adopted for these plants in the supplemental volume of the Century Dictionary.

CAMPHORA, CINNAMOMUM SAIGONICUM, CINNAMOMUM ZEYLANICUM, OLEUM CASSIE. The generic name, CINNAMOMUM of Blume, is antedated by 35 years, Noronha having given these plants, in 1790, the name Camphorina.

The proper combinations should be as follows:

The Camphor plant, CAMPHORINA CAMPHORA (Linné), n. comb. *Laurus Camphora* Linné Sp. Pl. 369, 1753.

Cinnamomum Camphora (Linné) T. Nees and Eberm. Handb. Med.-Pharm. Bot. 2, 430, 1831.

The Saigon Cinnamon. CAMPHORINA SAIGONICA, n. sp. The plant producing the Saigon Cinnamon has not as yet been definitely determined, but it is generally supposed to be an undescribed species. The bark is well described in the U. S. P. on pages 114 and 115, and I tentatively propose the above name for the species producing it.

The Ceylon Cinnamon. CAMPHORINA CINNAMOMUM (Linné), n. comb. *Laurus Cinnamomum* Linné, Sp. Pl. 369, 1753. *Cinnamomum Ceylanicum* Nees in Wall. Pl. As. Rar. 2, 74, 1831; 3, 32, 1832.

The Cassia Cinnamon. CAMPHORINA CASSIA (Linné), n. comb. *Laurus Cassia*, Linné, Sp. Pl. 369, 1753. *Cinnamomum aromaticum* Nees in Wall. Pl. As. Rar. 2, 74, 1831.

"Cinnamon" and "Cassia" are terms that have been applied from time immemorial to certain aromatic barks that have been used for ceremonial purposes, as well as in the medical and culinary arts. In founding the names *Laurus Cinnamomum* and *Laurus Cassia*, Linné adopted the descriptions of the Flora Zeylanica as his diagnoses of these species respectively. A reference to this Flora Zeylanica conclusively shows that Linné had in view and was describing the *Cinnamomi Cortex* and the *Cassie Liqnea Cortex* of the pharmacies. The former is the Ceylon Cinnamon and the latter is the Chinese Cassia. No other legitimate interpretation can be given to the Linnaean names, and they are so considered here.

CARYOPHYLLUS, OLEUM CARYOPHYLLI. If the clove and its allies are to be kept separate from *Eugenia*, as is insisted upon by many botanists, the proper generic name is *Caryophyllus* LINNÉ, and not *Jambosa* D. C., as maintained by Niedenzu in Engler and Prantl's Pflanzenfamilien III, 7, page 83. The binomial is *Caryophyllus aromaticus*, LINNÉ.

My thanks are due to Dr. F. V. Coville, of Washington, for calling my attention to the priority of the Linnaean name, and to the fact that *Caryophyllus* is the name used in the revised edition of the Century Dictionary under both "Clove" and "Caryophyllus"; also for pointing out that *Prunus communis* FRITSCH is used for the Almond in Bailey's Cyclopedia of Horticulture V, 2832, 1916.

COCAINA. The proper spelling of the generic name for the Coca plant is *ERYTHROXYLUM*.

KINO. The oldest generic name for the plant producing this product is *Lingoum* (kumphi) Adanson, and the proper binomial is *Lingoum Marsupium* (Roxburgh) O. K.

PRUNUS VIRGINIANA. The proper botanical designation for the tree producing this product is *Prunus Virginiana* LINNÉ, an older name than *Prunus serotina* Ehrhart. This interpretation of *Prunus Virginiana* LINNÉ is in accordance with that in vogue amongst most of the botanists of an earlier age, before Dr. Gray transferred the name to the Choke Cherry and adopted the later name for this, the Black Cherry. Incidentally, it is the name used in the earlier editions of the U. S. P. The Linnaean description is equally applicable to either species, for the petioles of the leaves

of either may or may not be glandular. The synonymy is entirely that of the Black Cherry. The specimen in the Linnæan Herbarium is that of the Choke Cherry, but this may be disregarded, as is generally done in so many other instances where the descriptions, including synonymy, and the specimens are at variance. Linnæus did not consider his herbarium specimens as "types"; in fact, it was often the custom among the early botanists to use for exchange, herbarium specimens that had been used as bases for descriptions of new species, trusting to time or luck to replace them with others; there is, therefore, no absolute surety that the specimen in the Linnæan Herbarium at the present time is the identical fragment from which Linnæus drew up his description. As Linnæus recognized but one American species in this group, it is more than probable that he considered the Black Cherry and the Choke Cherry as conspecific. To recapitulate: As the herbarium specimen is not a "type," and as there is no definite surety that it is the identical fragment that gave rise to the Linnæan description, and as the said description is so drawn that it is as applicable to the Black Cherry as to the Choke Cherry, there is left but the synonymy to interpret the species, *Prunus Virginiana* LINNÉ, and that proves it to be our common Black Cherry. Also, Du Roi, who was the first to segregate the Linnæan aggregate, used *Prunus Virginiana* for the Black Cherry and gave to the Choke Cherry the name *Prunus nana*, which, under the rules, must stand.

PETROSELINUM. In the Species Plantarum, Linné enumerated two species under his genus *APIUM*, viz.: *A. Petroselinum* and *A. graveolens* in the order named. *Apium* was adopted from the earlier botanists, and the first species enumerated was the one always considered as the type of the genus. *Apium Petroselinum* LINNÉ should, therefore, replace *Petroselinum sativum* Hoffman.

SABAL. The original spelling (*Screnca*) of the generic name has been adopted in Engler and Prantl's Pflanzenfamilien and should be taken up.

SACCHARUM. One of the sources of sugar is said to be *Beta vulgaris* LINNÉ, var. *Rapa* DUMORT. The proper citation for this variety is (Dumort) Ascherson, Dumort having used the name in a specific sense. The type of *Beta vulgaris*, LINNÉ, Sp. Pl. 222,

1753, is his var. *B. rubra*, as it is this variety which gives rise to the specific name which is typified by the common red beet of the gardens. It has received many varietal names, but the earliest one, in so far as I can determine, that applies to the Sugar Beet, that is, the form that is so largely cultivated for the manufacture of Beet sugar, is *Beta vulgaris*, LINNÉ, var. *altissima* (Rossig) J. A. Schultes.

SANTONICUM. The source of this is given as *Artemisia pauciflora* (Ledebour) Weber. The proper citation is "Weber" only; "(Ledebour)" should not be a part of the author citation, as Weber named the species ten years before Ledebour was born. There is some doubt as to the specific status of the plant producing Santonica; but the consensus of opinion seems to place it as a variety of *Artemisia maritima* LINNÉ. It might, therefore, be better to call it *Artemisia maritima* Linné, variety *Stechmanniana* Besser, which is the oldest varietal name for it.

SCILLA. The source for this drug is given as *Urginea maritima* (Linne) Baker. In the Species Plantarum, Linné included under his genus *Scilla* the elements of three different genera: one species from the old genus *Bulbus*, six species from the old genus *Hyacinthus*, and one species from the old genus *Scilla*. The single species from the old genus *Scilla* is the first one enumerated by Linné; also, since it is the plant that gave the name to the genus, it, *Scilla maritima*, LINNÉ, must be considered as the type of the genus. *Urginea* should give way to *Scilla* and the name should be *Scilla maritima* LINNÉ. The *Scilla* of Adanson (1763) and many subsequent authors not of Linné (1753), is *Stellastero* HEISTER (1763).

SENNA. There is no real reason why the Linnean name, *Cassia Senna* LINNÉ, should not be used for the Alexandria Senna, rather than the later one of Delile, *Cassia acutifolia*. The Linnean description and references are characteristic and unmistakable.

UVA-URSI. The proper name for the plant yielding this product is *Uva-Ursi Uva-Ursi* (Linné) Britton.

VERATRINA. The oldest name for the Cevadilla is *Skoinolon*. The binomial should be as follows: *Skoinolon officinale* (Chamisso and Schlechtendal), n. comb. *Veratrum officinale* Cham. and Schlecht. in Linnaea VI, 45, 1831.

BOTANICAL NOMENCLATURE OF THE N. F. IV.

In a Paper Appearing in the April Issue of the Circular, Mr. Farwell Discussed the Botanical Nomenclature of the Pharmacopoeia. He Here Gives a Similar Survey of the National Formulary IV.

BY OLIVER ATKINS FARWELL

A careful examination of the botanical nomenclature adopted in the National Formulary IV shows that it follows in part the Vienna Code and in part the American Code, with a strong leaning toward the latter as the predominant feature. The adoption of the trinomial system is to be deprecated, as it makes the authors express an opinion which they had never for an instant entertained. The trinomial system under the American Code is the method of expressing a subspecies, the "Code" not recognizing the rank of variety. Yet in every instance where the trinomial is used the author quoted did not publish a subspecies; he published a variety. Geographical names are decapitalized; as they are proper names they should be capitalized the same as is done with other proper names. The following notes and comments may be of service in the next revision:

AGARICUS. Derived from *Polyporus officinalis* Fries. This is not the valid name for the fungus producing the white agaric used in medicine. Winter, in the second edition of Rabenhorst's Kryptogamen Flora, uses the above name; Murrill, in North American Flora, uses the combination *Fomes Laricis* (Jacq.) Murrill; Hennings, in Engler u. Prantl's Pflanzenfamilien, adopts both *Fomes* and *Polyporus* as distinct genera, but unlike Murrill refers the white agaric to *Polyporus* as *P. officinalis*. The species of *Fomes* are, perhaps, by most authors regarded as species of *Polyporus*, but whether *Fomes* or *Polyporus* the oldest and valid specific name is *Laricis*. The proper name under *Polyporus* is *P. Laricis* (Jacq.) Scopoli.

ASARUM. Hyphenated words are rapidly going out of favor, the word being written as either one word or two distinct words; "snakeroot" is the most generally accepted way of writing the word, not "snake-root."

CACTUS GRANDIFLORUS. The botanical origin is given as *Cactus grandiflorus* Linné, with the synonym *Cereus grandiflorus* Miller. These names certainly appertain to the drug known com-

mercially as "cactus grandiflorus," but they are only synonyms and should not be used, especially the Linnæan name, for the plant producing the drug has not been classed in the genus *Cactus* by any botanist for nearly a century and a half. The proper name for this drug is *Selenicereus grandiflorus* (Lin.) Britton and Rose. In the third line of the description on page 275 the words "each about 2 mm" would be more accurate if changed to read "5 mm or less," and the word "spines" after "flexuous" should be changed to "bristles." It may not be out of place to note here that a related Mexican species, the *Selenicereus pteranthus* (Link and Otto) Britton and Rose, has been used as a substitute. This drug is distinguished from the true by the absence of the long bristles from the tufts of spines.

CENTAURIUM. The drug is indicated as being derived from the *Erythraea Centaurium* (Linné) Persoon. The oldest generic name is *Centaurium* Hill. There is an older *Centaurea* Linné, but as the ending is different and it belongs to a very different family of plants, no confusion can arise from accepting Hill's generic name for these plants, as has been done in our local manuals. The proper botanical designation is *Centaurium Centaurium* (Linné) W. F. Wight.

CHIRATA. In the U. S. P. 7th Revision the botanical source of this drug was given as *Swertia Chirata* Hamilton. It was changed in the 8th Revision on my advice, to *Swertia Chirayita* (Roxb.) Hamilton, which was retained, also on my advice, in the present, 4th edition of the National Formulary. But this combination has never been properly published, and Hamilton did not use this form of spelling. The specific name has been spelled in various ways by various authors, as will be seen from the synonymy given below. The proper designation is as follows:

SWERTIA CHIRAYITA (Roxb.) Farwell (nov. comb.).

Gentiana Chirayita Roxb. in Flem. in As. Res. XI p. 167 (1810) and in Flem. Cat. Ind. Medic. Pl. 21 (1810).

Gentiana Chirata Wall Pl. As. Rar. III, 33, t. 252 (1832).

Gentiana Chirayta Roxb. Fl. Ind. II, 74, 1832.

Gentiana Cherayta R. Fleming in C. B. Clarke Ed. Roxb. Fl. Ind. 264, 1874.

Swertia Chirayta (Roxb.) Karsten Deut. Fl., 1025, 1880-1883.

CORNUS. Those species of *Cornus* in which the inflorescences are surrounded by a corolla-like involucre are better considered as constituting a distinct genus. The proper name for the plant under this view is *Cynoxylon floridum* (Linné), Raf.

CORYDALIS. The proper spelling of the generic name is *Bikukulla*.

CYPRIPEDIUM. The drug is indicated as being obtained from three species, *Cypripedium hirsutum* Miller, *C. pubescens* Willd., and *C. parviflorum* Salisb.

Cypripedium hirsutum. This name of late years has had a varied career. It was first published by Philip Miller in 1768. Henrietta G. Fox used it in 1895 for the large yellow-flowered ladies' slipper and it was later transferred to the large and showy white-flowered *Cypripedium Regine* Walter, which name should supersede *C. hirsutum* Miller in the Formulary. Miller described his *C. hirsutum* as a plant one and a half feet in height, with oblong-oval, deeply veined leaves and reddish-brown flower, flowering in May. In so far as my acquaintance with *Cypripedium* goes, Miller's description can apply to only one—the *C. acaule* Ait. This plant may be found in dry, sandy woods, in rocky woods, in rich, moist woods, and in peat bogs; it ranges in height from 3 or 4 inches to 22 inches, well above the limit assigned by Miller. It is certain that Miller would never have called the yellow flowers of *C. pubescens* or the characteristically white flowers of *C. Regine* "reddish-brown," which color just suits that of the flowers of *C. acaule*. The time of flowering of the latter also agrees with the time given by Miller, while the flowering time of the former is in July, long after *C. acaule* has ceased to bloom. The proper name for the moccasin flower is:

FISSIPES HIRSUTA (Miller) Farwell (nov. comb.).

Cypripedium hirsutum Miller Gard. Dict. Ed. 8, No. 3, 1768.

Cypripedium acaule Ait. Hort. Kew III, 303, 1789.

The rhizomes and roots of this species could be used as well as those of the others allowed; they probably form part of the commercial drug.

Cypripedium pubescens and *C. parviflorum*. The oldest name for the large, yellow-flowered ladies' slipper is *Cypripedium bulbosum* Miller, l. c. No. 2. Linnæus had another and older species of the same name, but as that belongs to a very different genus

Miller's name is the valid one, and the small, yellow-flowered ladies' slipper is *Cypripedium bulbosum* Miller, var. *parvillorum* (Sabisb.) Farwell.

DROSERA. There is some confusion existing regarding the nomenclature to be adopted for some of the species of *Drosera*. If *Drosera Anglica* Huds. were adopted instead of *Drosera intermedia* Hayne, the other names remaining as given, the result would be more in accordance with the rules of priority.

EVONYMUS. The proper spelling for this generic name is *Evonymus*.

EUPHORBIA PILULIFERA. The proper name for the plant from which this drug is produced is *Euphorbia hirta* Linné; or if considered as a genus distinct from true *Euphorbia*, *Chamaesyce hirta* (Linné) Millspaugh.

GOSSYPII CORTEX. In *Gossypium Barbadense* Linné, the specific name, a geographical one, is capitalized, as it should be; but this is an oversight of the proof-reader, as the intention was to decapitalize all such names. They should be recapitalized.

KAVA. This drug is said to be derived from *Piper Methysticum* Forster. The name is not tenable for this plant because of an earlier and valid *Piper Methysticum* Linné filius; also the authority cited should have been Forster filius. The proper names and synonymy for the two species are as given below. Both are known as "ava," and as there is a very noticeable difference in the physical appearance of the roots of different lots of drug it is possible that both species enter into the make-up of the commercial drug.

Piper Methysticum, Linné filius, Suppl. 91, 1781; and Lam. Ill. I. p. 81 (1791).

Piper latifolium Linne filius Suppl. 468, 1781, and Forster filius Prod. 5, 1786.

Macropiper latifolium Miq. Syst. Pip. 218, 1843-4.

The species of the National Formulary is:

PIPER ESCULENTUM (Raf.) Farwell (nov. comb.).

Piper Methysticum Forster filius Pl. Escul. 76, 1786, and Prod. 5, 1786 non Linne, filius 1781.

Methysticum esculentum Raf. Sylva Tellur. 85, 1838.

Macropiper latifolium Miq. in Linnaea XX (1847) 130.

Methysticum Methysticum (Forster) Lyons Plant Names 301, 1907.

It is to be noted that C. DeCandolle in the *Prodromus*, vol. 16, part 1, page 354 (1869), and Hooker and Jackson in the *Index Kewensis*, vol. 2, p. 142 (1895), quote *Macropiper Methysticum* Hook. and Arn., Bot. Beech. Voy. p. 96, as a synonym of this species. These citations are erroneous, as Hooker and Arnott used the combination *Piper Methysticum*. There is a considerable difference of opinion among botanists as to the generic status of these plants, some retaining them in the genus *Piper* and others in *Macropiper*, the oldest name for which is *Methysticum* Raf. *Sylva Tellur*, 85, 1838. Under this genus the first species above with synonymy as there given would be:

Methysticum Methysticum (Linné filius) Farwell (nov. comb.); and the other, *Methysticum esculentum*, Raf.

KRAMERIA. *Krameria Ixina* Linné should be *Krameria Ixine* Linné. "Ixine" is an old generic name, and was used as a specific name by Linnæus in 1758. In the *Species Plantarum*, 1762, it appeared as "Ixina," perhaps a typographical error. "Ixina" has been in general use, but the older spelling should be restored.

KOLA. Said to be derived from several species of *Cola* Schott and Endlicher. *Cola* is not tenable for this genus, there being several older names, the oldest being *BICHEA* Stokes. The most important species yielding kola is:

BICHEA ACUMINATA (Beauv.) Farwell (nov. comb.).

Sterculia acuminata Beauv. Fl. d'Ow 1, t. 24, 1804.

Bichea solitaria Stokes Bot. Mat. Med. II 565, 1812.

Cola acuminata Schott and Endl. Meletem, 33, 1832.

LEPTANDRA. *Leptandra* is said to be derived from "*Veronica Virginica* Linné." This plant is often considered to be generically distinct from true *Veronica* under the name of *Leptandra* Nuttall; the oldest generic name, however, is *Veronicastrum* Heister in Fabricius, 1759. The proper nomenclature, according to rules of priority, for the plants producing this drug, is:

VERONICASTRUM VIRGINICUM (Linné) Farwell (nov. comb.). Comb.).

Veronica Virginica Linné Sp. Pl. 9, 1753, and—

VERONICASTRUM (Lin.) Farwell, var. *LANCEOLATUM* Farwell (nov. comb.).

Callistachya Virginica (Lin.) Raf. var. *lanceolata* Farwell Ann. Rpt. Mich. Acad. Sci. XVII, 176 (Reprint 1916).

MATICO. The drug is said to be obtained from the *Piper angustifolium* Ruiz et Pavon. The name is not tenable for this species, as it is the valid name for the species better known as *Piper consanguineum* Kunth. Matico is derived from *Piper granulosum* Ruiz et Pavon, which is the valid name for the species.

MELILOTUS. Said to be derived from "*Melilotus officinalis* (Linné) Lamarck"; it should read *Melilotus Melilotus-officinalis* (Linné) Ascherson and Græbner.

OLEUM AURANTII, AMARI AND FLORUM. The botanical origin should read *Citrus Aurantium* Linné; the "*amara*" between the words "*Aurantium*" and "Linné" is superfluous.

OLEUM BERGAMOTTE. The words "Linné" and "variety" or its abbreviation "var." should be inserted between the words "*Aurantium*" and "*Bergamia*"; Wight and Arnott described a variety, not a subspecies.

OLEUM CARDAMOMI. The proper name for this is *Amomum Cardamomum* Linné. If the later generic name is to be used the correct citation would be *Elettaria Cardamomum* (Linné) Maton.

OLEUM MYRICE. The proper author citation for *Pimenta acris* is (Swartz) Kostel, not Wight.

PERSIO. "(Fam. Parmeliaceæ)" should be inserted after "lichens."

PETROSELINI RADIX. *Petroselinum Petroselinum* (Linné) Karsten is the valid designation of this product. *Petroselinum hortense* Hoffmann also has precedence over *P. sativum*.

PHYTOLACCA. The proper and valid name is *Phytolacca Americana* Linné.

PIMENTA. The valid designation for this is *Pimenta Pimenta* (Linné) Karsten.

SASSAFRAS MEDULLÆ. The valid name for this product is *Sassafras Sassafras* (Linné) Karsten.

SUCCUS CITERI. The words "Linné" and "variety" or "var." should be inserted between "*Medica*" and "*acida*." Bonavia named and described a variety, not a subspecies. The word "*medica*" is a proper name derived from *Medica*, and should be capitalized; also to distinguish it from "*medica*" referring to use as a medicine.

TEREBINTHA LARICIS. The proper designation of the species producing this drug is *Larix Larix* (Linné) Karsten.

VERBASCI FOLIA. Besides *Verbascum Thapsus* Linné, this drug is allowed to be derived from "other species of verbascum." Since the genus contains 200 or more species of wide variation in the physical, and probably in the therapeutic, properties of the leaves, it would seem to be more appropriate to limit the drug to *Verbascum Thapsus*.

XANTHOXYLI FRUCTUS. The generic name should be spelled with an initial Z instead of an X.

In order to restore the earliest family name used and to have them all end in "aceæ" the following changes should be made :

Fagaceæ	to	Castaneaceæ.
Moraceæ	to	Lupulaceæ.
Polygonaceæ	to	Persicariaceæ.
Euphorbiaceæ	to	Tithymalaceæ.
Terebinthaceæ	to	Pistaciaceæ.
Rhamnaceæ	to	Zizyphaceæ.
Sterculiaceæ	to	Cacaoaceæ.
Araliaceæ	to	Hederaceæ.
Umbelliferæ	to	Umbellataceæ.
Oleaceæ	to	Jasminaceæ.
Loganiaceæ	to	Strychnaceæ.
Labiataæ	to	Labiataceæ.
Rubiaceæ	to	Aparinaceæ.
Cucurbitaceæ	to	Bryonaceæ.
Compositæ	to	Compositaceæ.
Leguminosæ	to	Leguminaceæ, Krameriaceæ and Lomentaceæ.
Rosaceæ	to	Rosaceæ, Pomaceæ, and Drupiferaceæ.
Celastraceæ	to	Arillataceæ.
Ericaceæ	to	Monotropaceæ.

For assistance in bibliography my thanks are due to Dr. N. L. Britton, of the New York Botanical Garden; Dr. F. V. Coville, of Washington, D. C.; Dr. J. A. Nieuwland, of Notre Dame, Ind., and to Miss Edith Wycoff, of Cincinnati, O.

Mr. O. A. Farwell, author of this article, and of a similar article on the botanical nomenclature of the Pharmacopœia IX which appeared in the April issue of the *Circular*, was born in Dorchester, Mass., December 13th, 1867. When five years of age his family removed to Michigan, where, in the public schools and under the guidance of private tutors, he received his early education.

His father died in 1881, and at the age of fourteen, the future botanist found himself thrown suddenly on his own resources. For a period of eleven years he tried his hand variously at farming, wood-cutting, engineering and teaching, and finally, in 1892, he entered the employ of Parke, Davis & Co., and at the close of the year was appointed assistant to the botanist and librarian. The following year he took on the added duties of drug inspector, and more lately, in 1909, relinquished the librarianship in order that he might devote his undivided attention to botany and pharmacognosy. He has published a number of papers relative to the flora of Michigan and the histology of pharmaceutical drugs.

Mr. Farwell has been an active member of the Torrey Botanical Club of New York, for more than twenty years, and is a charter member of the Michigan Academy of Science and of the Botanists of the Central States. He is a member of the American Pharmaceutical Association, the American Joint Committee on Horticultural Nomenclature, and of the National Geographic Society.

He is a member of the following patriotic and social societies: Sons of the American Revolution, New England Society of Detroit, Society of the Descendants of Pilgrim John Howland, and the Society of Mayflower Descendants.

He is fond of hunting, fishing, traveling and reading, and is a bachelor. When asked the why and wherefore of this latter he modestly admits that he is still too young, not having reached the age of discretion.

[The following comments on Jalap should have appeared as a portion of Mr. Farwell's article on botanical nomenclature of the United States Pharmacopœia, published in the April issue.—Editor *The Druggists Circular*.]

JALAPA. The proper botanical designation for this drug is *Exogonium Jalapa* (Nuttall and Coxe) Baillon. Nuttall was the first author to name the Jalap of commerce and medicine; he named it *Ipomœa Jalapa* in the *Journal of the American Medical Sciences*, Vol. 5 for 1829, p. 300, 1830. There is an older use of this combination, *Ipomœa Jalapa*, Pursh, so that Nuttall's name, if the plant is to remain in *Ipomœa*, as some authors maintain, must give way to the next oldest, which is *Ipomœa Purga* (Wenderoth) Hayne. If maintained as distinct from *Ipomœa*, as most authors contend, Nuttall's earlier name is available and should be adopted.

NATIONAL FORMULARY BOTANICAL NOMENCLATURE.

**The Author Here Supplements a Paper by Him, Appearing in The
Druggists Circular for May of Last Year, and Adds a Criticism
of the Naming of Two Drugs not in the Official Books.**

BY OLIVER ATKINS FARWELL.

API FRUCTUS. Linné included two species, *A. Petroselinum* (the Parsley) and *A. graveolens* (the Celery) under his genus *Apium* in the first edition of the *Species Plantarum*. As the Parsley is the type species of the genus *Apium*, the Celery, if distinct, must be given another name. This was done by Adanson in 1763 in the *Families des Plantes*, where he gave it the name *Celeri*. The proper name is *Celeri graveolens* (Linné) Britton.

BERBERIS. Said to be derived from the rhizome and the roots of species of the section *Odostemon* Rafinesque. Rafinesque published the name *Odostemon* as of generic rank and not as the name of a section of a genus. It antedates Nuttall's name, *Mahonia*, by a year. The oldest sectional name is *Mahonia*. DE CANDOLLE, in J. A. and J. J. Schultes' *Systema*, VII, pt. 1, 17, 1829. This name should replace *Odostemon*.

BRAYERA. The botanical source of this drug is given as *Hagenia Abyssinica* (Bruce) Gmelin, Cusso, Bruce Trav. V, 1, 22 and 23, 1790, is the older name. The proper combination is *CUSSO ABYSSISCA* (Gmelin), n. comb.

Hagenia Abyssinica Gmelin, Syst. 2, 613, 1791.

ECHINACEA. *Echinacea angustifolia* DE CANDOLLE should not be classed as a synonym of *Brauneria pallida* (Nuttall) Britton: it should be maintained as a distinct species under the name *Brauneria angustifolia* (De Candolle) Heller.

GALANGA. Said to be derived from *Alpinia officinarum* Hance. In the *Species Plantarum* 1753, Linné adopted the genus *Alpinia* from Royen and listed but one species thereunder, the *Alpinia racemosa*, by later botanists referred to *Renealmia* Linné filius. As there was but one species listed under *Alpinia* by the elder Linné, the generic name *Alpinia* must naturally be accepted for it in preference to the later name of the younger Linné. As this species and the one producing the drug Galangal are not con-

generic, the latter manifestly has been referred to the wrong genus. The oldest generic name available for the Galangal is *LANGUAS KOENIG*, in Retz, Observ. III, 64, 1783. The proper combination is:

LANGUAS OFFICINARUM (Hance), n. comb.

Alpina officinarum Hance, in Journ. Linn. Soc. XIII, 6, 1873, and Journ. Bot. 175, 1873. This combination appears under Galangal in the Century Dictionary, IV, p. 2432 (1911), but I am unable to say who is responsible for the binomial.

HELIANTHEMUM has been restricted and the sections *HALIMUM*, *TUBERARIA* and *FUMANA* have been restored to generic rank. The species producing the drug fall within the genus *HALIMUM* but it is not the oldest available name; *Trichasterophyllum* WILDENOW has several years priority. *Helianthemum major* also enters into the makeup of the commercial drug and both species should be allowed as its source. They are:

TRICHASTEROPHYLLUM CANADENSE (Linné), n. comb.

Cistus Canadensis Linné, Sp. Pl. 526, 1853.

TRICHASTEROPHYLLUM MAJOR (Linné), n. comb.

Lechea major Linné, Sp. Pl. 90, 1753.

PETROSELINUM. Said to be derived from *Petroselinum sativum* Hoffman. It should be *Apium Petroselinum* LINNÉ. As this species is the type of the genus *Apium*, this name, which is the oldest, should be used, rather than the later one.

PULSATILLA. The group of plants of which the pulsatilla is typical seems to be as distinct from *Anemone* as is *Hepatica*, the Liverwort. The proper names under this genus are *Pulsatilla Pulsatilla* (Linné) Karsten, *Pulsatilla pratensis* (Linné) Miller, and *Pulsatilla patens* (Linné) Miller, var. *Wolfgangiana* (Besser) Ledebour. If the latter is to be considered as distinct from the European plant then it should be *Pulsatilla hersutissima* (Pursh) Britton; if retained as at present under *Anemone*, the American species should be *Anemone patens* LINNÉ, var. *Wolfgangiana* (Besser) Koch, or *Anemone hirsutissima* (Pursh) MacMillan. The American plant is better considered as a variety of the European than as a distinct species.

JUGLANDACEÆ should give way to the older family name of *Nuculaceæ*.

My thanks are due to Dr. A. B. Lyons, of Detroit, for calling

to my attention the fact that the combination *Saertia Chirayita* (Roxburgh) had been made by himself in the first edition of *Plant Names*, 360, 1900; and to Dr. F. V. Coville of Washington that the combination *Bichea acuminata* (Swartz) had been made in 1909 by W. F. Wight in the *Century Dictionary*, XI, 271, and in 1912 in the *Bulletin of the Bureau of Plant Industry*, No. 233, 60.

In my papers on the Botanical Nomenclature of the United States Pharmacopœia IX and of the National Formulary IV, I have used the French form of the name of the Swedish botanist in order to be in conformity with the usage of those works. In future editions it would be better, it seems to me, to use the Latin form, Linnaeus, or the simple Swedish form, Linn, in order to be more in conformity with general usage.

In my capacity as a member of the Committee on Non-official Standards of Drugs and Chemicals of the American Pharmaceutical Association it fell to my lot to write up the description of several non-official drugs.

In my researches along these lines it became evident that two of the drugs investigated were known under specific names which are not the oldest ones applied to them, and, therefore, are not the proper names under which they should be known according to the laws of priority. One of the plants is the Verbenaceous *Premna Taitensis* Schauer. Its native names are "Yoro" and "Awalho." It is a native of Fiji and other Pacific islands, among them the Tonga Islands, which give the pharmaceutical name "Tonga" to the drug. The correct name is:

PREMNA ARBOREA (Forst. f.), n. comb.

Scrophulariodes arborea Forst f. Prodr. No. 528, p. 91, 1786.

Premna Taitensis Schauer in D. C. Prodr. XI p. 638, 1847.

(?) *Lomatia cymosa* Sol. in Seem. Fl. VII, 187, 1865-73.

It is most remarkable that the authors of the *Index Kewensis* listed the last above named synonym *Lomatia cymosa*, Sol., as the name of a valid species of the Protiaceous genus, *Lomatia*, R. Br., with which it is in no wise connected. There is an older *Premna arborea* Roth, but as that name is but a synonym of *Gmelina arborea* Roxb., it can not invalidate the use of the specific name "arborea" for this species.

The other species is that coniferous tree of northwestern Africa, known as Arar Tree or Sandarac Tree and produces the drug Sandarac. It belongs to that division of the Pine family which includes the Cypress, Cedar, and Juniper.

It was first described as *Thuja articulata* Vahl. in Symb. Bot. II, p. 96, 1791. Ventenat renamed it *Callitris quadrivalvis* in Decad. 10, 1808. As Vahl's name is the older one, the proper name should be *CALLITRIS ARTICULATA* (Vahl), n. comb. There is an older *C. articulata* Hort. for a horticultural form of *C. rhomboidea* R. Br., but as it is only a synonym of the latter it can not invalidate the use of the specific name "articulata" for the Sandarac Tree.

Studies from the Research Laboratory.
Parke, Davis & Co.
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SISYRINCHIUM BERMUDIANA.

BY OLIVER ATKINS FARWELL.

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Many botanists have in the past considered the pale-blue-flowered *Sisyrinchium Bermudiana* L., of the Atlantic coast, and the violet-blue *S. iridioides* Curtis, of Bermuda, to be conspecific and have united them under the Linnæan name. Philip Miller, who cultivated both, side by side, considered them to be amply distinct and described them separately in the Gardeners Dictionary in 1768 but applied the Linnæan name to the Bermuda plant and renamed the Atlantic coast species as *S. angustifolium*. William Curtis, who, like Miller, knew both plants, also considered them to be distinct and in the Botanical Magazine, plate 94, named the Bermuda plant *S. iridioides*; the date of the title page of volume 3 of the Botanical Magazine is 1790 but the printed date on the plate itself is September 1, 1789; the publication of the binomial must, therefore, date from that of the plate, 1789. Modern botanists follow the interpretation of Philip Miller by applying the name *Sisyrinchium Bermudiana* L. to the plant that is endemic in the Bermudas, but this is contrary to the laws of priority as expressed in both the Vienna and American codes. Both of these species were described and illustrated by Plukenet in the *Almagestum* under his genus *Sisyrinchium*; likewise by Dillenius in *Hortus Elthamensis* under the Tournefortian genus *Bermudiana*. Linnæus in the *Species Plantarum*, page 954, 1753, combined both species under the binomial *Sisyrinchium Bermudiana*, thus preserving to science both of the old generic names under each of which the species had previously been known. The specific name *Bermudiana* perpetuates an old generic name and cannot be considered as having been given to the species as a geographical name to indicate the nativity of the species; had that been the idea actuating Linnæus he in all probability would

have given it the name *bermudiense*, adopting it from Plukenet, *providing he had intended the Bermudian plant to be the type of the species*. But Hemsley has already shown (*Journal of Botany* 22: 108-110, 1884) that Linnaeus in all probability had never seen the plant from Bermuda. As a matter of fact he made the Bermuda plant his var. *B* and considered it to be of such small categorical importance that he did not give to it even a varietal designation. That he intended the Virginia plant to represent typically his *S. Bermudiana* is clearly proved by the fact that all references to it were enumerated under his specific name and description while those referring to the Bermuda plant were grouped under his unnamed variety *B*, and by the fact, which is still more to the point, that the explanatory note with its fuller description was drawn entirely from his "Planta *a*," *i.e.*, the Virginia plant. A careful study of all the evidence seems to indicate that:

1. Linnaeus probably never saw the plant from Bermuda.
2. The specific name *Bermudiana* perpetuates an old generic name and was not used as a geographical name to indicate the origin of the species; this view *per se* would prevent the adoption of the Bermuda plant as the type of the species.
3. The Linnaean descriptions (diagnosis and footnote) are based upon the plant from Virginia, which must therefore be taken to be the type of the species.
4. The plant from Bermuda should be known under the first name applicable to it, *S. iridioides* Curtis.



Studies from the Research Laboratory.

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NOTES ON THE MICHIGAN FLORA.

BY OLIVER ATKINS FARWELL.

(Department of Botany, Zarke, Davis & Company, Detroit, Michigan.)

This paper is the first of a series that will record from time to time such notes on rare or interesting plants of Michigan that may be brought to light as the result of field work continued from year to year, together with such remarks on the last edition of the Michigan Flora, by Dr. Beal, as may be deemed necessary to bring the nomenclature in that work up to present standards. This series, to all intents and purposes, is a continuation of "*The Contributions to the Botany of Michigan*" begun in the Asa Gray Bulletin.

POLYPODIACEAE.

ASPLENIUM ANGUSTIFOLIUM, Mx.

A very graceful fern, local in distribution and not very plentiful when found. A very peculiar feature about this fern is the profusion of sterile fronds as compared with the fertile; clump after clump may be examined without discovering a single fertile frond, and occasionally a station for this fern may produce nothing but sterile fronds. No. 1531, Rochester, July 4, 1896; No. 4542½, Stevens, July 29, 1917.

FILIX NOVEBORACENSIS (Linn.) Farwell.

This fern seems to be one of our rare ones; it is closely related to the prolific Swamp Shieldfern but is confined to rich woods while the latter, as the name indicates, is found everywhere in preferably open, swampy or boggy grounds. No. 4588, Rochester, September 12, 1917.

FILIX GOLDIEANA (Hooker) Farwell.

In moist woods. A large broad fern with large, rather distant pinnæ. Only occasional. No. 1532, Rochester, July 4, 1896; No. 4182, Utica, June 6, 1916.

FILIX BOOTHII (Tuckerman) Farwell.

This species was found in a tamarack swamp near Oxford; the only other place in Michigan known to me is in Keweenaw County. These plants agree perfectly with the one represented by plate 69 in Eaton's Ferns of North America. No. 553, Keweenaw County, August 29, 1887; No. 4767, Oxford, October 16, 1917.

OPHIOGLOSSACEAE.

BOTRYCHIUM DISSECTUM, Spreng. var. OBLIQUUM (Muhl.) Clute.

In open woods and in fields that have not long been denuded of their woody growth. Scarce. No. 4808, at Junior near Royal Oak, October 28, 1917.

BOTRYCHIUM DISSECTUM, Spreng. var. LONGATUM (Gilb. & Haber.) Farwell.

In similar situations and sometimes associated with the preceding variety but much rarer. No. 4809, Junior, October 28, 1917.

PINACEAE.

JUNIPERUS COMMUNIS, Linn.

This is the typical arborescent form of the species and is quite rare here; it is to be looked for on high, dry grounds, as indeed are the varieties, although one of them may be found on low and in rather swampy places. No. 4739, Oxford, October 11, 1917.

JUNIPERUS COMMUNIS, Linn. var. DEPRESSA, Pursh.

A low shrub forming large mats of various shapes, by means of the decumbent stems and branches, from which the branchlets ascend to a height of three feet or less.

Leaves and fruit much as in the pieces. Common in Oakland County on hillsides and even extending down into swampy grounds. No. 2074, Rochester, May 12, 1909.

No. 2074a, Geddis, August 21, 1909; No. 2843, July 14, and No. 2892, July 28, 1912, Parkedale; No. 4239, June 29, 1916, Cass Lake.

JUNIPRES COMMUNIS, Linn. var. MONTANA, Ait.

A low shrub forming large or small circular mats three feet or so in height. While the mats formed by this variety are to all appearances much the same as in the preceding variety, yet upon close examination it is apparent that they are formed somewhat differently; in most cases the branches radiate from a short central trunk for shorter or longer distances and then ascend in the manner of a rosette; the leaves are shorter, broader, strongly curved at base, and then erect or nearly so, being sub-appressed; the berries are larger than in the other forms above enumerated. Although this is not a *trailing* shrub it can scarcely be placed otherwise than with this variety. No. 45, August 4, 1883, and No. 3074, August 22, 1912, Keweenaw County.

GRAMINACEAE.

HOLCUS SORGHUM, Linn.

Waste grounds at Detroit, No. 1416, August 18, 1883.

Holcus saccharatus, Linn.

Waste grounds at Detroit, No. 1957, October 2, 1905.

Holcus saccharatus, Linn., var. TECHNICUS (Koernicke), n. comb. *Andropogon Sorghum* Brot. var. *technicus* Koernicke, in Hackel in DC. Mon. Phaner, VI, 508-9, 1889.

Waste grounds at Detroit, No. 2504, September 7, 1911.

HOLCUS DURRA, Forsk.

Waste grounds at Detroit, No. 3928, October 26, 1914.

HOLCUS DURRA, Forsk, var. ÆGYPTIACUS (Koernicke), n. comb. *Andropogon Sorghum* Brot. var. *Ægyptiacus* (Koernicke) Hackel l. c., 516-7.

Waste grounds at Detroit, No. 1958, October 2, 1905. The Sorghums, like Oats, Wheat and Rye, are frequently found in waste places. Sometimes the Stations are destroyed by the improvements in real estate that are continually taking place, and again they will spring up in other places. They seem not to take a strong foothold in the local flora, but their appearance is rather due to the result of seed being thrown out with other rubbish at periodical times of cleaning up.

THE GENUS PANICUM IN MICHIGAN.

Since the publication of Beal's Michigan Flora in 1904, the genus *Panicum* has been the subject of intensive and extensive studies in field, herbarium and library, with the result that many new species have been published. Dr. Beal enumerated 24 species; the number now known to be in Michigan is 38, an increase of over 50%; while the ranges of distribution of 10 others either cross Michigan or run along her borders. As may naturally be expected, there are a number of changes in nomenclature. Below is a revised list showing the necessary changes in nomenclature, and such additions as have been enumerated by Hitchcock and Chase in their monograph of the *North American Species of Panicum* published in 1910 as Volume 15 of the Contributions from the United States National Herbarium, and others that I have since found or that have been segregated from the old aggregates.

PANICUM DICHOTOMIFLORUM, Mx.

Dr. Beal lists this as *P. proliferum* Lam., but the plant is not that species. No. 4101, September 23, 1915, at Oakwood, and No. 4812, Detroit, November 15, 1917.

PANICUM FLEXILE (Gattinger) Scribner.

Number 893, Orion, August 29, 1895; No. 893a, Detroit, August 29, 1912.

PANICUM PHILADELPHICUM, Bernh.

No. 3214b, Detroit, October 15, 1912. First time reported, I believe.

PANICUM CAPILLARE, Linn.

No. 536, Keweenaw Co., August 20, 1887; No. 536a, Belle Isle, August 18, 1892.

PANICUM MILIACEUM, Linn.

No. 1414, Detroit, August 18, 1893; No. 1414a, Keweenaw Co., August, 1894.

PANICUM VIRGATUM, Linn.

No. 1928, Island Lake, July 16, 1905; No. 2139½, St. Clair Flats, September 27, 1909; No. 3055, Algonac, August 11, 1912; No. 3809, Parkedale, July 30, 1914. No. 3055½, Algonac, August 11, 1912 (var. *conferta*, Vasey); No. 2126½, Detroit, August 21, 1909 (var. *elongata*, Vasey).

PANICUM AGROSTOIDES, Spreng.

Washington, Macomb Co. Dr. Cooley. Only station known in the State.

PANICUM STRICTUM, Pursh.

No. 3984, Rochester, June 20, 1915. Listed by Beal as *P. depauperatum*, Muhl.

PANICUM PERLONGUM, Nash.

No. 115, Keweenaw Co., July 18, 1890; No. 155a, Palmer Park, June 16, 1898; No. 2210, Algonac, June 11, 1911; No. 3970, Parkedale, June 13, 1915; No. 3987, Parkedale, June 25, 1915. Not reported by others in so far as I am aware.

PANICUM LINEARIFOLIUM, Scribn.

No. 597, Keweenaw Co., September 10, 1887; No. 3986, Parkedale, June 25, 1916; also at Dearborn. Agricultural College, 1888, Lake. This and the preceding are segregates from the old *P. depauperatum*.

PANICUM WERNERI, Scribn.

Flint, Dr. Clark (Field Museum Herbarium).

PANICUM BOREALE, Nash.

No. 643, Keweenaw Co., August 8, 1888.

PANICUM DICHOTOMUM, Linn.

No. 2107, Rochester, August 15, 1909; No. 4275, Palmer Park, July 2, 1916; also Dearborn, 1917. Port Huron in 1899 and 1909, Dodge; and Grand Beach Springs, Hill, 84 in 1908.

PANICUM BARBULATUM, Mx.

Port Huron, Dodge in 1899; Park Lake, Wheeler 17.

PANICUM DUMUS, Desv.

Listed by Dr. Beal as *P. maculatum* Ashe.

PANICUM LINDHEIMERI, Nash.

Port Huron, Dodge in 1909.

PANICUM MERIDIONALE, Ashe.

No. 3831, Parkedale, August 9, 1914; Port Huron, Dodge in 1909; Twin Lakes, Wheeler 24, 28; Magician Lake, Umbach, 2155.

PANICUM ALBEMARLENSE, Ashe.

Cass County, Pepoon in 1904.

PANICUM IMPLICATUM, Scribn.

No. 597a, August 12, 1888; No. 597b and No. 613a, August 16, 1888, Keweenaw Co.; No. 1610¹/₂, Detroit, July 12, 1898; No. 2860, Parkedale, July 14, 1912; also Dearborn. Port Huron, Dodge in 1909; Port Alger, Wheeler, in 1895.

Listed by Dr. Beal as *P. dichotomum* var. *gracile* at least in part; this variety also included, in all probability, what is now known as *P. meridionale*.

PANICUM HUACHUCAE, Ashe.

No. 643b, Belle Isle, July 21, 1892; No. 2825, Algonac, July 7, 1912; Howard Terrace, Wheeler in 1899. Was listed by Dr. Beal as *P. Columbianum*; possibly also a part of *P. dichotomum* var. *commune*.

PANICUM HUACHUCAE, Ashe, var. FASCICULATUM (Torr.) Hubbard.

No. 597c, Keweenaw Co., August 16, 1888; No. 597d, Belle Isle, August 31, 1892; No. 1382, Detroit, June 24, 1893; Port Huron, Dodge in 1909; Grand Beach Springs, Hill, 83 and 85, in 1908. Listed by Dr. Beal as a variety of *P. dichotomum*.

PANICUM TENNESSEENSE, Ashe.

No. 3985, Parkedale, June 25, 1915; Grand Beach Springs, Hill 86 in 1908; Petoskey, Hill 162 in 1878.

PANICUM PRECOCIUS, Hitch and Chase.

No. 3462, Parkedale, June 15, 1913. No. 3816, Rochester, August 9, 1914; Port Huron, Dodge in 1909. Was listed by Dr. Beal as *P. pubescens*.

PANICUM SUBVILLOSUM, Ashe.

No. 642, Keweenaw Co., August 18, 1888; No. 2210½, Algonac, June 11, 1911; No. 3788, July 26, 1914. Was listed by Dr. Beal as *P. nitidum*.

PANICUM VILLOSISSIMUM, Nash.

Carleton, Wheeler in 1890.

PANICUM PSEUDOPUBESCENS, Nash.

Saginaw Bay, Morris 240 in part; Twin Lakes, Wheeler in 1900.

PANICUM TSUGETORUM, Nash.

Port Huron, Dodge in 1909; Twin Lakes, Wheeler 82; Port Austin, Morris A240 in part.

PANICUM SPHAEROCARPON, Ell.

No. 1388, Detroit, July 3, 1893; Grand Beach Springs, Hill 88, 90 in 1908; Magician Lake, Umbach, 2153.

PANICUM MULTIFLORUM, Ell.

No. 2127a, Royal Oak, August 24, 1909; Washington, Dr. Cooley. Listed by Dr. Beal as *P. polyanthes*.

PANICUM MACROCARPON, Torr.

No. 1610, Detroit, July 12, 1898; No. 3969½, Parkedale, June 13, 1915. Also at Lothrop and at Dearborn. Very common in Wayne Co. Listed by Dr. Beal as *P. Scribnerianum*.

PANICUM LEIBERGII (Vassey) Scribn.

Hansens Island, Dodge in 1899.

PANICUM xanthophysum, A. Gr.

No. 764, Keweenaw Co., July 18, 1890.

PANICUM UMBROSUM, Le Conte.

Muskegon, Wheeler 19.

PANICUM COMMUTATUM, Schult.

No. 1425, Detroit, August 25, 1893; Agricultural College, Wheeler, 1890.

PANICUM CLANDESTINUM, Linn.

No. 3814, Parkedale, August 9, 1914; No. 3889, September 13, 1914; Port Huron, Dodge, in 1904 and in 1909.

PANICUM LATIFOLIUM, Linn.

No. 1378, Detroit, June 22, 1893; No. 3821, Parkedale, August 9, 1914; No. 4256, Cass Lake, June 29, 1916; Port Huron, Dodge in 1909. Was listed by Dr. Beal as *P. macrocarpon*.

PANICUM BOSCH, Poir.

No. 1920, Detroit, June 29, 1905; No. 3187, Parkedale, June 15, 1913. Was listed by Dr. Beal as *P. Porterianum*.

PANICUM BOSCH, POIR. var. MOLLE (Vasey) Hitch and Chase.

No. 4011, Algonac, July 25, 1915. Not before reported from the state, I believe.

In the 10th annual Report of the Michigan Academy of Science Dr. Beal listed additional species. Of these *Panicum Asheri* Pearson is *P. umbrosum*; *P. lanuginosum* Ell. is probably some one of the species listed above but can scarcely be the species of Elliott; *P. laxiflorum* Lam. is not the species of that author but probably either *P. boreale*, Nash or *P. Nalapense*, H. B. K.; *P. pubescens* Lam. is probably some one of the species enumerated above but not the species of LaMarck.

Below is a list of species of *Panicum* that may confidently be expected to be found, sooner or later, in Michigan by reason of the fact that their known ranges of distribution either include some part of Michigan or pass very closely to her borders.

Panicum Gattingeri, Nash

Panicum elongatum, Pursh.

Panicum anceps, Mx

- Panicum verrucosum*, Muhl.
Panicum Xalapense, H. B. K.
Panicum microcarpon, Muhl.
Panicum lucidum, Ashe.
Panicum spretum, Schult.
Panicum scoparioides, Ashe.
Panicum oligosanthos, Schult.

MUHLENBERGIA MEXICANA (Lin.) Trin.

More or less common along sandy or gravelly banks that are generally moist or damp; No. 2128½, August 25, 1909, Starr; No. 3876, September 7, 1914, Parkedale; No. 4395, August 22, 1916, Bloomfield; No. 4606, September 16, 1917, Bloomfield.

MUHLENBERGIA MEXICANA (Lin.) Trin. var. COMMUTATA (Scribn.) Farwell.

- Agrostis filiformis*, Willd. Enum. Hort. Berol. 1, 95, 1809.
Agrostis foliosa Hort. Willd. l. c.; as a synonym.
Muhlenbergia foliosa Trin. Gram. Unifl. 190, 1824.

In similar situations but less frequent. No. 906, August 29, 1896, Orion; No. 906a, September 9, 1897, Palmer Park; No. 1656, September 12, 1899, Belle Isle; No. 4561, September 9, 1917, Monroe Piers.

A good deal of confusion has arisen through certain botanists taking it for granted that the *Agrostis filiformis* Muhl. is the same thing as Willdenow's species of the same name; but a careful comparison of Muhlenberg's description with that of Willdenow will show that these two authors were describing different species. The former described a species with an *unawned* floret of about the same length as the equal glumes; the latter had a species with an *awned* floret longer than the unequal glumes. The former is what has been passing as *M. foliosa*, Trin. or *M. ambigua* Torr.; the latter is the awned state of *M. Mexicana*. As there is already a valid *Muhlenbergia filiformis*, the species must be known by the next available name, which is *M. ambigua*, Torr. Torrey's species is, however, the awned state, while that of Muhlenberg was the unawned condition and this may be known as:

MUHLENBERGIA AMBIGUA, Torr., var. FILIFORMIS (Muhl.), n. comb.

- Agrostis filiformis*, Muhl. Gram. 66, 1817.

Agrostis lateriflora Mx. var. *filiformis* (Muhl.) Torr. Fl. I, 86, 1824.

MUHLENBERGIA DIFFUSA (Muhl.), n. comb.

Agrostis diffusa Muhl. l. c. 64, Not Host.

Agrostis sylvantica Torr. l. c. 87, Not Lin.

Muhlenbergia umbrosa Scribn. Rhodora 9, 20-1, 1907.

There is an earlier *M. diffusa* Willd., but as this is but a synonym of *M. Schreberi*, Gmel., there is no valid obstacle to restoring the earliest name given to the species. No. 4530, October 11, 1917, Oxford; No. 4675, October 7, 1917, Rochester.*

REBOULEA PALLENS (Spreng.) Farwell var. MAJOR (Torr.) Farwell.

In low, moist grounds near Dearborn; not before reported from the state in so far as I am aware; No. 4515, July 8, 1917.

PANICULARIA NERVATA (Willd.) O. K.

The typical plant is strict, about 1 foot in height, with erect unexpanded oblong panicle not over 6 inches long; spikelets $1\frac{1}{2}$ - $2\frac{1}{2}$ lines long by $1\frac{1}{4}$ - $1\frac{3}{4}$ lines wide, ovate or oblong, bright green, or when fully mature with a slight purplish tinge along the apical border of the florets, of which there are 4 to 7 to the spikelet. Not common, generally in rather dry situations. No. 4502a, June 17, 1917, Parkedale; No. 645, August 8, 1888, Keweenaw County.

Panicularia nervata (Willd.) O. K., var. FILIFORMIS, n. var. Plant larger, two or three feet or more in height, panicle longer, linear or filiform, 6 to 10 inches in length, erect or slightly curved, branches appressed or subappressed, spikelets smaller, 1 line long by $\frac{1}{2}$ line in width, livid or pale green, distant; lower glume $\frac{1}{4}$ line long, 2nd glume $\frac{3}{8}$ line long; simulates *P. melicaria* (Mx.) Hitchc. Low wet grounds, Dearborn, No. 4511 $\frac{1}{2}$, July 8, 1917.

Panicularia nervata (Willd.) O. K., var. PURPURASCENS, n. var.

*The state with awless flowering glume may be known as *Muhlenbergia diffusa* var. *attenuata* (Scribn.), n. comb. *Muhlenbergia umbrosa* Scribn. subsp. *attenuata* Scribn., l. c. 21.

Intermediate between the species and the var. *filiformis* as to size of plant; expanded panicle usually ample, branchlets generally drooping, spikelets purplish throughout. The commonest form, in meadows; No. 4495½, June 10, 1917, Utica; No. 517, August 10, 1887, Keweenaw County; No. 2786, June 30, 1912, and No. 3489, June 15, 1913, at Parkedale; No. 517a, July 2, 1892, Belle Isle.

CYPERACEAE.

Cyperus ferax, Rich.

Recorded as *C. speciosus*, Vahl, from Hubbardston and Flint. At Monroe Piers, Farwell and Billington. This species seems to be one of the rare ones here; No. 4568, September 9, 1917.

Cyperus strigosus, Linn., var. *compositus*, Britt.

Found in abundance in low grounds at Dearborn. Not before recorded from Michigan; No. 4666, September 29, 1917, Dearborn; No. 4592, September 15, 1917, River Rouge.

Stenophyllus capillaris (Linn.) Britt.

Found by Mr. Billington at the Zoo Park near Royal Oak. Rare in Michigan, No. 4559, September 8, 1917.

Carex Deweyana, Schw., var. *STRICTA*, n. var. Low, about six inches in height, yellowish green leaves and culms of about the same length and rigidly erect. On dry sterile hills and fields, Keweenaw County, No. 603, September 12, 1887. The typical species is a bright green plant of moist woodlands with long weak culms several times longer than the leaves, usually lying flat on the ground or very nearly so. No. 603a, August 12, 1888, Keweenaw County; 603b, June 20, 1895, Mackinac Island; No. 4498, June 10, 1917, Utica.

Carex lupulina, Muhl. var. *Bella-villa* (Dewey) Bailey.

Pistillate spikes scattered, prigynia spreading, horizontal or nearly so. Rare, No. 4793, October 26, 1917, Rockwood, Farwell and Gladewitz. Not before recorded for Michigan.

JUNCACEAE.

Juncus tenuis, Willd. var. *anthelatus*, Wiegand.

More robust than the species, inflorescence longer, and the flowers secund. The first time reported from the State. No. 4530, July 14, 1917, Dearborn.

Juncus interior, Wiegand, var. UNIFLORUS, n. var.

Very slender, almost capillary, four or five inches in height, inflorescence reduced to a single, sessile flower. No. 4533 $\frac{1}{2}$, July 14, 1917, at Dearborn, Billington and Farwell.

Juncus interior, Wiegand, var. BILLINGTONII, n. var.

Slender, four or five inches in height, inflorescence umbellate, somewhat similar to that of *Juncoides pilosum* var. *Michiganense*, of three or four slender, spreading branchlets, each ending in a single flower. Named for Mr. Cecil Billington of Detroit. No. 4533 $\frac{1}{3}$, July 14, 1917, Dearborn, Billington and Farwell.

Juncoides pilosum (Linn.) Coville var. MICHIGANENSE, n. var.

Root leaves half evergreen, more or less persistent, 8 to 12 inches long by 2-4 lines wide and longer than the stems at flowering time; stem leaves about 3, 7 to 20 lines long by 1-2 wide; umbell mostly simple but occasionally some of the slender somewhat unequal branches with a second remote flower; perianth segments, *dark chestnut brown, shining*; No. 4428 $\frac{1}{2}$, May 13, 1917, Bloomfield. In the typical European species the valves of the pod are abruptly contracted into oblong, broadly obtuse tips, while in the American plant the valves are triangular, with straight edges, and acute or acutish.

Juncoides pilosum (Linn.) Coville var. SALTUENSE (Fernald) n. comb.

Luzula saltuensis Fernald, Rhodora, V, 195, 1903.

This differs from the preceding variety in having the perianth segments of a pale brown color; No. 2559, May 19, 1912, Parkedale Farm.

Polygonatum boreale, Greene, var. MULTIFLORUM, n. var.

A robust form $2\frac{1}{2}$ to 4 feet in height, with the lower peduncles bearing four flowers, the central ones three flowers, and the upper two and one; No. 4488, June 5, 1917; Walled Lake, Farwell and Chandler.

Polygonatum melleum, N. Sp.

Plant about two feet or less in height; leaves ovate, larger ones three or four inches long, glabrous beneath, many nerved but none except the midrib conspicuously larger than the others, sessile to distinctly short petiolate, not amplexicaule; peduncles short, about $\frac{3}{4}$ inch or less, lowermost four flowered, reducing to one on the uppermost; pedicels unequal, the longest about the length of the peduncle; flowers deep honey yellow, 5 to 6 lines long, cylindrical; anthers and free portion of the filament of equal lengths or the latter a trifle longer and minutely but densely pubescent, the pubescence, in the fresh flower, being distinctly noticeable to the unaided eye. On sandy grounds in open thickets at Algonac; No. 3974, June 16, 1915.

SALICACEAE.

Salix pedicellaris, Pursh.

This swamp or bog willow has been restored to specific rank, as it is no longer considered to be a variety of the European *S. Myrtilloides*. It differs from the variety *hypoglauca*, Fernald, which is the more common form, in having broader leaves green on both sides. No. 87, June 22, 1884, Keweenaw County; the variety occurs at Walled Lake, No. 4480, June 5, 1917, and in Keweenaw County, No. 2050, June, 1908.

Salix serrissima (Bailey) Fernald.

This is the rare autumn fruiting willow. The usual time for flowering of this species is so much later than for the other species of the genus that the superabundance of surrounding foliage renders the spikes so inconspicuous that they

are generally overlooked. About the only time that it is at all noticeable is in the autumn when the bursting pods make the plant a very conspicuous object in the landscape, No. 1753¹/₂, Lakeville, September 2, 1901; No. 2741, June 23, 1912, Parkedale; No. 4762, October 16, 1917, Oxford.

CORYLACEAE.

Betula pumila Lin., and its varieties.

The low Swamp Birch is very variable in size and shape of leaf and extent of pubescence even on the same plant. In a Tamarack swamp near Walled Lake my attention was attracted to a form of the species that appeared to be less pubescent than usual, most of the branches being entirely glabrous, showing a bright, reddish brown bark and deeper green, *glabrous leaves*; this is the var. *glaber*, Regel. At Oxford was another form, the young leaves of which, when pinched, felt sticky with exuding resin. The resinous character proves it to be the var. *glandulifera*, Regel.

Betula pumila, Lin.

No. 227, June 20, 1885, Keweenaw County; No. 227a, May 30, 1895, Orion; No. 2560, May 19, 1912, Parkedale; No. 4217, June 15, 1916, Pontiac; No. 4451¹/₂, Oxford, May 20, 1917.

Betula pumila, Lin., var. *glandulifera*, Regel.

May 20, 1917, Oxford, No. 4453¹/₂.

Betula pumila, var. *glaber*, Regel.

The early glabrous form, not resinous; No. 4486, June 5, 1917, Walled Lake.

Another small shrub Birch, not pubescent, but with glandular, warty branches, is *Betula glandulosa*, Mx. No. 32, July 13, 1883, Keweenaw County; No. 3088, August 26, 1912, Calumet. A dwarfer form with rounder leaves is var. *rotundifolia* (Spach) Regel. No. 4823¹/₂, August 1904, Keweenaw County.

CASTANEACEAE.

Fagus grandifolia, Ehrh. var. *Caroliniana* Fern. & Rehd.

Dr. Beal lists *F. Americana* Sweet in the Michigan Flora. All that I have seen in southern Michigan is of the variety *Caroliniana*. Probably the Beech of the Upper Peninsula is typical *F. grandifolia*, as that region is so far north of the present known range of the variety as to preclude the possibility of the northern Beech belonging to it. No. 865, May 1, 1896, Highland Park; No. 3256, October 27, 1912, Parkedale; No. 4634, September 23, 1917, Tecoma; No. 4447¹/₂, May 19, 1917, Farmington. (Flowering branches, 2-6 inches high from lateral buds on an exposed root.)

Quercus lyrata, Walt.

This, the Swamp Post Oak, occurs on the banks of Paint Creek, north of Rochester. Mr. Brotherton discovered it many years ago and first pointed it out to me in 1896. As it is not recorded in the Michigan Flora, I presume it was not reported by Mr. Brotherton. No. 4583, September 12, 1917.

ULMACEAE.

Celtis occidentalis, Lin.

The Hackberry is quite frequent in southern Michigan. No. 2120, August 21, 1909, Geddes; No. 4754, October 14, 1917, Redford. Dr. Beal does not list the var. *crassifolia* (Lam.) A. Gr., but it is frequent, nevertheless, in Michigan. It is distinguished from the species by its leaves being very scabrous above and by the pubescent branchlets. No. 4747, October 14, 1917, at Redford; No. 4800, October 26, 1917, at Rockwood.

LUPULACEAE.

Morus rubra, Lin.

The native Red Mulberry is found on river banks and in rich woods. There are not many of the wild trees now left and they do not bear much resemblance to the cultivated form.

The leaves are often a foot wide by 16-18 inches in length exclusive of a two-inch petiole, generally unlobed or occasionally 2 or 3 lobed. No. 4771, October 20, 1917, at Redford and No. 4790, October 26, 1917, at Rockwood. As an escape from cultivation: No. 1821, July 13, 1904, Grosse Pointe Farms, and No. 3960, May 31, 1915, Detroit.

URTICACEAE.

Parietaria Pennsylvanica, Muhl.

Credited to VanBuren County by H. S. Pepoon in "Additions to the Michigan Flora." I have collected it at Geddes, No. 2124, August 21, 1909; Farmington, No. 4532, July 29, 1917; and Rockwood, No. 4789, October 26, 1917, Farwell and Gladewitz.

ARISTOLOCHIACEAE.

Asarum Canadense, Linn.

In a previous paper, I made the remark that *A. Canadense* probably was not found in Michigan but that all our wild Gingers should be referred to *A. acuminatum* and *A. reflexum*. Such a statement was unwarranted and was based on an accidental mixture of specimens and labels which at the time had escaped detection. The species is quite common in woods in southeastern Michigan and probably elsewhere. No. 1617, August 25, 1898, Keweenaw County; 1617a, June 2, 1899, Belle Isle; 1617b, September 9, 1899, Birmingham; No. 3310, May 4, 1913, Parkedale.

No. 287, August 1, 1887, Keweenaw County, is the variety *acuminatum*, Ashe.

No. 1668, June 13, 1900, Belle Isle; No. 1668a, June 9, 1901, Birmingham; No. 3389, May 25, 1913, Parkedale; No. 4448, Farmington, May 19, 1917, and No. 4469, June 2, 1917, St. Clair Heights are the variety *reflexum* (Bickn.) Robinson.

A. Canadense, Linn. var. **AMBIGUUM** (Bickn.) n. comb.

A. reflexum (Bickn.) var. *ambiguum*, Bickn. Bul. Torr. Bot. Cl. XXIV, 535, 1897.

Differs from the var. *reflexum* in the long acuminate lobes bearing the same relation to that variety as the var. *acuminatum* does to the specific type. The color of the lobes as compared with typical *A. Canadense* is very much brighter, being of a distinctly reddish tinge while that of the species is dark purplish brown, and the white parts of the inner side of the tube are more conspicuous as they are more extensive. No. 1618, August 25, 1898, Keweenaw County; 1618a, May 19, 1907, Detroit; No. 2591, May 27, 1912, Algonac; No. 4501½, June 10, 1917, Utica.

PERSICARIACEAE.

Polygonum acre, H. B. K.

A common plant in wet places. No. 1343, September 23, 1892, and 1343a, September 27, 1893, Belle Isle. A small form has been named *P. acre* var. *leptostachyum*, Meisn. No. 2007, September 12, 1896, Belle Isle; No. 4620, September 23, 1917, Tecoma. A large form has been described as *P. punctatum* var. *robustius* Small, Bul. Torr. Bot. Cl. XXI, 447, 1894, and may be known as *P. acre* var. **ROBUSTIUS** (Small) n. comb. No. 2006, September 12, 1906, Belle Isle; No. 4621, September 23, 1917, Tecoma.

Polygonum scandens, Linn.

A twining perennial in moist thickets. Fruiting calyx green with scarious, crisped margins, 5 lines long or somewhat longer; achene 2½ lines long by 1½ wide. No. 4796, October 26, 1917, Rockwood, Farwell and Gladewitz. No. 904, August 29, 1895, Orion; 904a, September 18, 1895, Belle Isle.

Polygonum dumetorum, Linn.

High climbing; fruiting calyx 4 lines or less long, pale brown or yellowish white with entire margins; achene about 1¾ lines long by 1 wide. Strikingly different from the preced-

ing and by far the commoner species in southeastern Michigan. No. 4560, September 8, 1917, Zoo Park, Royal Oak; No. 1688, October 2, 1900, Detroit; No. 2981, August 14, 1912, and No. 1417, September 13, 1914, Algonac.

Rumex Patientia, Linn., var. *Kurdicus*, Boiss.

Roadsides at Rochester, Mich. Well established. The conspicuous grain on the fruiting calyx proves this to be the var. *Kurdicus*. Not listed in the "Flora." No. 2092, June 30, 1909.

BLITACEAE.

Beta vulgaris, Linn.

The common Red Beet of the gardens has become adventive in waste places at Detroit. No. 1441, September 25, 1893.

Kochia scoparia (Linn.) Schrad.

Commonly cultivated for its bright color during the autumn months and known as Mexican Fireweed. Has escaped from cultivation. No. 4596, September 15, 1917, River Rouge; No. 1851, August 23, 1901, Detroit.

Chenopodium vulvaria, Linn.

A low plant with small broadly ovate leaves, whole plant exhaling a fetid odor. No. 1432, August 31, 1893, Detroit.

Chenopodium rubrum, Linn.

Similar to *C. album* but not mealy and the calyx is slightly fleshy and red. Detroit, No. 3215, October 20, 1912.

Atriplex hortensis, Linn.

The Garden Orache, extensively cultivated in many parts of the world and used as Spinach. Waste grounds at Detroit, No. 1412, August 18, 1893, and No. 3198, September 29, 1912.

AMARANTHACEAE.

Amaranthus hybridus, Linn, Sp. Pl. 909, 1751.

Amaranthus chlorostachys, Willd., Hist. Amarant. 34, t. 8. f. 19, 1790.

Somewhat similar to *A. retrofractus*, but more slender and

flexuous, smoother and greener, bracts longer awned. Not so common. No. 4558½, September 8, 1917, Royal Oak; No. 1330, September 7, 1892, Belle Isle.

Amaranthus hybridus, Linn., var. *SANGUINEUS* (Regel), n. comb.

Amaranthus hypocondriacus Linn. Sp. Pl. 991, 1753.

Amaranthus paniculatus Linn. var. *sanguineus* Regel. Flora XXXII, 164, March, 1849, not Moq. May, 1849.

Amaranthus paniculatus, Linn. var. *erythrostachys* Moq. in DC., Prodr. XIII, Pt. 2, 259, May, 1849.

Amaranthus hybridus, Linn., var. *Hypochondriacus* (Linn.). Robinson, Rhodora X, 32, 1908.

Commonly cultivated in gardens as Princess Feather. An escape and well established in some places. No. 156, June 19, 1894, and No. 3189, September 19, 1912, Detroit.

Amaranthus hybridus, Linn., var. *DENSUS* (Regel), n. comb.

Amaranthus paniculatus Linn. Sp. Pl. 1406, 1763.

Amaranthus paniculatus a sanguineus c densus, Regel Flora XXXII, 164, March, 1849.

Amaranthus paniculatus var. *purpurascens* Moq. in DC. Prodr. XIII, Pt. 257, May, 1849.

Amaranthus hybridus var. *paniculatus* (Linn.) Uline & Bray, Mem. Torr. Bott. Cl. V, 145, 1895.

The purple Amaranth of Gardens. Frequent as an escape. No. 4564, September 9, 1917, Monroe Piers; No. 1489, October 19, 1894, and No. 3193, September 26, 1912, Belle Isle.

Amaranthus hybridus, Linn., var. *cruentus* (Linn.) Moq.

Amaranthus cruentus Linn. Syst. X, 1269, 1759.

Amaranthus sanguineus, Linn. Sp. Pl. 1407, 1763.

An escape from cultivation; waste grounds, Detroit. October 17, 1915, No. 4130.

Acnida tamariscina var. *concatenata* (Moq.) Uline & Bray of the "Flora" becomes *A. tuberculata*, Moq. var. *subnuda*, Watson. No. 1703, October 3, 1900, Detroit.

Acnida tamariscina var. *tuberculata* (Moq.) Uline & Bray of the "Flora" becomes *A. tuberculata*, Moq. No. 1701, October 2, 1900, Detroit; No. 1704a, August 21, 1909, Geddes; No. 4554, September 8, 1917, Royal Oak. This and the next were also observed at Monroe Piers, September 9, 1917.

Acnida tuberculata, Moq. var. *prostrata* (Uline & Bray) Robinson.

No. 4555, September 8, 1917, Royal Oak; No. 1483, September 24, 1894, Belle Isle.

ILLECEBRACEAE.

Anychia polygonoides, Raf.

Listed in the "Flora" as *A. dichotoma* Mx. No. 2120¹/₂, August 21, 1909, Geddes; No. 2125, same station and date, is *A. Canadensis* (Linn.) B. S. P. Both these species evidently are rare in Michigan, as but two stations for this and three for the former are given by Dr. Beal.

ALSINACEAE.

In the "Flora" *Alsine*, Linn., is adopted for *Stellaria*, Linn.

ALSINE Linn. Sp. Pl. 272, 1753, is the proper generic name for that genus of plants that is better known as *SPERGULARIA* or *TISSA*. The *Alsine borealis* becomes *Stellaria borealis*, Rigel, var. *isophylla*, Fernald; the *Alsine borealis alpestris* (Fries) Britt. becomes *Stellaria borealis*, var. *alpestris*, A. Gr. As the varietal name of Gray was based on plants collected by Dr. Robbins in the Lake Superior region his name *alpestris* should be adopted rather than the later *floribunda* of Fernald. The reference to his variety by Dr. Gray of the European *S. alpestris*, Fries, and *S. Fenzlii*, Regel, is merely a case of misidentification and cannot invalidate Gray's varietal name. The other species of *Alsine* will be known under *Stellaria* with the same specific appellations respectively as listed under *Alsine*. *S. borealis* Rigel, var. *isophylla*, Fernald No. 361, June 1, 1886, Keweenaw County. *S. borealis* var. *alpestris*, A. Gr. No. 362, June 1, 1886, Keweenaw County.

Stellaria media (Linn.) Cyril.

A common dooryard weed and a pest of cultivated grounds.

Said to be naturalized from Europe; No. 252, July 5, 1885 Keweenaw County; 252a, June 23, 1891, Ypsilanti; No. 252b, September 30, 1892, Detroit; No. 2673, June 11 1912, Parkedale.

It is found, however, along the banks of streams in woods, etc., where nothing but native plants are found. In these situations there is no doubt in my mind but that it is as certainly native as any other plants amongst which it grows. No. 4432, May 19, 1917, Farmington.

Stellaria media (Linn.) Cyr., var. *SUCCULENTA* (Linn.) n. comb.

Holosteum succulentum Linn. Sp. Pl. 88, 1753, et. Amoen. Acad. III, 21, 1756.

Plant somewhat succulent; stems procumbent and often 3 feet or more long forming dense mats of inextricably tangled herbage; upper leaves sessile, elliptical, often $1\frac{1}{2}$ -2 inches long by one-third as wide. In low grassy hollows in open copses, associated with native *Asters*, *Heliopsis*, etc., and probably as native as the plants in whose company it is growing. No. 4753, October 14, 1917, Redford.

Tissa rubra (Linn.) Britt. becomes *Alsine rubra* (Linn.) Crantz, and *Spergularia media* (Linn.) J. & C. Presl, *Alsine media* (Linn.) Crantz.

Moehringia, Linn., *Ammodenia*, Patrin, and *Minuartia*, Linn., are united with *Arenaria*, Linn. or kept distinct from it according to the individual views concerning the limitations of genera, of those authors who are studying these plants.

The consensus of their opinions, however, is for keeping them distinct from *Arenaria*. As there already is a valid *Minuartia stricta* (Swz.) Hiern, which is considered by American botanists to be distinct from *Arenaria stricta*, Mx. (Fl. I, 274, 1803), the latter must take a new specific name under *Minuartia*; the next available name is *Alsine Michauxii* Fenzl, Verbreit. Alsin. 18, 1833 and the com-

bination becomes *MINUARTIA MICHAUXII* (Fenzl) n. comb. No. 934, August 29, 1895, Orion; Nos. 2645, June 9, 1912, 3435, June 8, 1913, and 3475, June 15, 1913, Parkedale.

Silene dichotoma, Ehrh.

Common in the vicinity of Rochester. In Gray's Manual this species is said to have a 5-ribbed calyx, but the plants at Rochester have the calyx very prominently 10-ribbed. No. 2836, July 14, 1912, Parkedale; No. 4556, September 27, 1917, Rochester.

Silene Anglica, Linn.

Rather a rare plant in Michigan. No. 158, August 24, 1904, Detroit.

NYMPHAEACEAE.

In *Rhodora* for July, 1916, Mr. Conard has very conclusively shown the genera *Nymphaea*, Linn. and *Nuphar*, Sm. should be retained for the White Water Lilies and the Yellow Pond Lilies respectively.

RANUNCULACEAE.

Pulsatilla patens (Linn.) Mill. var. *Wolfgangiana* (Bess.) Ledeb.

P. hirsutissima (Ph.) Britt.

The American Pasque Flower is scarcely distinct specifically from the European. This is included under *Anemone* in Gray's Manual, where *Hepatica* is maintained as a distinct genus; but *Pulsatilla* has just as good claims for generic distinction as *Hepatica*. No. 2048¹/₂, May, 1908, Keweenaw County.

Anemone Riparia, Fernald.

Similar to *A. Virginiana* in foliage, but it has large white petaloid sepals which are persistent year after year. Wooded banks at Rochester; No. 4329¹/₂, July 15, 1916.

Anemone Virginiana, Lin. f. *leucosepala*, Fernald.

Similar to the specific type but differing in having petaloid sepals of a creamy white color. The sepals are not pure white as in *A. riparia*; they are smaller and obovate, while those of the latter are broadly oval. The petaloid character of the sepals is not reliable, at least in this vicinity. On July 14, 1917, a large patch of this form, No. 4533, was discovered on the banks of a small ravine near Dearborn; it answered in all respects to the description as published by Mr. Fernald excepting as to size of anthers, which in this plant had a wider range of length, 0.75 mm. to 1.5 mm.. Upon visiting the same place a year later the same patch had the usual small sepals characteristic of the specific type; only two small plants had the petaloid sepals, the flowers of the other plants having reverted to the normal form for the species. Have also collected this form at Detroit, No. 1731, July 19, 1901.

BERBERIDACEAE.

Caulophyllum thalictroides (Linn.) Mx. var. *GIGANTEUM*, n. var.

Much larger in all its parts: panicle 3 or 4 inches long; leaflets $2\frac{1}{2}$ –4 inches long by $1\frac{1}{2}$ wide, broadly obovate to ovate, more generally rounded at base; petals longer, more tapering to the base, usually purplish. No. 4450, May 19, 1917, Farmington.

In the specific type the leaflets are about half the size of the above, generally cuneate at base, and the petals smaller, not tapering to the base, and of a greenish color. No. 4449, May 19, 1917, Farmington; No. 1238a, May 19, 1917, Detroit; No. 1238, May 21, 1892, Ypsilanti.

CRUCIFERACEAE.

Bursa Bursa-pastoris (Linn.) Britt. var. *INTEGRIFOLIA* (D. C.) n. comb.

Capsella Bursa-pastoris var. *integrifolia* DC. *Syst. II.* 384, 1821.

All the leaves oblanceolate and entire, capsule truncate at apex, whole plant weak, branched and straggling. Woods at Farmington; No. 4541, July 21, 1917.

SINAPIS, Linn.

The Charlock, listed by Dr. Beal under *Brassica*, should properly be referred to this genus under the name *Sinapis arvensis*, Linn. No. 630a, August 1, 1890, Keweenaw Co.; 630g, August 12, 1891, Ypsilanti; No. 630h, July 16, 1892, Belle Isle; No. 4507, June 28, 1917, Rochester.

There is a form of the species which is much more hispid than the type, the pods particularly being densely hispid with retrorse bristles. These two forms bear the same relation to each other as do *Sisymbrium officinale* and its var. *leiocarpum*, only in the latter instance it is the specific type that has the hispid pods. Linnaeus described the form with hispid pods as *S. orientalis* Amoen. Acad. IV: 280, 1759. It may therefore be known as *Sinapis arvensis*, Linn. var. *ORIENTALIS* (Linn.) n. comb. No. 2089, June 30, 1909, Rochester.

The White Mustard, *Sinapis alba*, Linn., is not at all common in the State. No. 630, July 31, 1888, Keweenaw Co.; 630c, June 21, 1894; and No. 2505, September 7, 1911, Detroit.

Barbarea verna (Mill.) Asch.

This species of Yellow Cress was found at Oxford, May 20, 1917. It was found in low grassy lands alongside of road gutters. No. 4454.

SEDACEAE.

Sedum ternatum, Mx.

Credited to Michigan in Gray's Manual but not included in the "Flora." It was first found in abundance at Southfield by the late Mr. Chandler. No. 4502½, June 16, 1917.

ROSACEAE.

Opulaster Opulifolius (Linn.) O. K. var. *INTERMEDIUS* (Rydb.), n. comb.

Opulaster intermedius Rydb. in Britt. Man. 492, 1901.

Mature fruit pubescent. Runs gradually into the species. Nos. 2738, June 23, 1912, 2778, June 30, 1912, and 3549, October 5, 1913, Parkedale; No. 3719, July 4, 1914, Stoney

Creek; No. 4133, October 22, 1915, Detroit; No. 3983, June, 1915, Rochester; the species has been collected at Keweenaw County; No. 114, June 30, 1884.

Sorbaria Sorbifolia (Linn.) A. Br.

An escape from cultivation. Common on roadsides near Stoney Creek.

Spiraea salicifolia, Lin. is an Asiatic species not found in America except as a rare escape from cultivation. We have two species.

Spiraea alba, Du Roi, with oblanceolate to oblong leaves. No. 3783, July 26, 1914, Algonac; No. 2915, July 28, 1912, Parkedale; 115b, July 21, 1893, Belle Isle; 115d, August, 1904, Keweenaw County; 4700, October 7, 1917, Orion; also at Oxford.

Spiraea alba, Du Roi, var. *LANCEOLATA* (T. & G.), n. comb.

Spiraea salicifolia Linn. var. *lanceolata* T. & G. Fl. N. A. I. 415, 1840.

Leaves narrowly oblanceolate. No. 115, June 30, 1884, Keweenaw County; 115c, July 23, 1902, Detroit; 3781, July 26, 1914, Algonac; also at Oxford.

Spiraea latifolia (Ait.) Borkh.

Leaves broadly oblanceolate or obovate. No. 115a, July 23, 1891, Ypsilanti.

Potentilla recta, Linn.

Listed in the "Flora" as *P. sulphurea* Lam.

It is spreading rapidly and is common in places. No. 4529 July 14, 1917, Dearborn; No. 1161, June 27, 1891, Ypsilanti; 1161a, July 31, 1909, Detroit.

Potentilla Anserina, Linn.

Common in places. No. 181, August 22, 1884, Keweenaw County; 181a, July 16, 1892, Belle Isle.

Potentilla Anserina, Linn. var. *sericea*, Hayne.

Differs from the species in having the leaves silvery silky on both sides; No. 4785½, October 21, 1917, Lothrop.

Potentilla Anserina, Linn. var. *Groenlandica*, Tratt. (var. *grandis* T. & G.)

A very luxuriant form of the species usually found in low, rich meadow land. No. 3667, June 12, 1915, Detroit.

POMACEAE.

Pyrus Aucuparia (Linn.) Ehrh.

The European Rowan or Mountain Ash was discovered in a Tamarack Swamp at Oxford. Possibly originating from seed dropped by some bird; it seemed to be in a thriving condition. No. 4455, May 20, 1917. Has also escaped at Ypsilanti, No. 1169, July 21, 1891, and on Belle Isle, 1169a, August 13, 1904.

CRATAEGUS Linn.

The *Cr. brevispina* of the "Flora" is *Cr. Brockwayae*, C. S. Sargent. No. 116, June 30, 1884, and No. 3060, August 22, 1912, Keweenaw County.

Crataegus Crus-galli, Linn.

This species is quite common in southeastern Michigan. Nos. 1333, 2597, and 3231.

Crataegus Crus-galli, Linn., var. *Prunifolia* (Marsh.) Loud. Corymbs and under surface of leaves more or less pubescent; leaves very large, often over 3 inches wide. The name, *Prunifolia*, dates from H. Marshall, who described the pubescent form. Synonyms are: *Mespilus Prunifolia* Marsh, Arbust. 90, 1885; French Ed. 110, 1888; *Crataegus Prunifolia* Bosc; DC. Prod. 11, 627, 1827; *Crataegus per-similis*, C. S. Sarg.; and *C. Farwellii*, C. S. Sarg., Mich. Geol. Surv. Rep. 1006, p. 519 (1907); *C. Crus-galli X macracantha* W. W. Eggl Rhodora, N. 76, 1908. This variety is found occasionally. No. 1760, Sept. 25, 1901, Belle Isle.

Crataegus Crus-galli, Linn., var. *ATTENUATA* (Ashe) n. comb.

C. attenuata Ashe, Jour. Elisha Mitch. Soc. XIX pt. 1, 30, 1903.

C. Bartramiana C. S. S. Proc. Acad. Phil. LVII, 582, 1905.

C. Crus-galli. var. *Prunifolia* W. W. Eggl. Rhodora X 75, 1908 not of Loudon.

Similar to the var. *Prunifolia* but leaves and corymbs glabrous. According to Eggleston this is *Mespilus Prunifolia* Poir. *Crataegus Prunifolia* Pers. Eucher. 1137, 1807, is probably the same thing. No. 4672, October 7, 1917, Rochester.

Crataegus Arduennae, C. S. S.

Detroit, October 13, 1904, No. 1884.

Crataegus punctata, Jacq. var. *aurea*, Ait.

An occasional form in which the fruit is yellow; No. 4749, October 14, 1917, Redford; 2135, September 6, 1909, and 3540, October 5, 1913, Rochester; 3551, October 5, 1913, Parkedale.

Crataegus punctata, var. *canescens*, Britt.

An occasional form in which the leaves and corymbs are densely canescent. No. 2021, June 16, 1907, Detroit.

Crataegus Taetrica, C. S. S.

Detroit, May 30, 1912, No. 2595.

Crataegus opulens, C. S. S.

Detroit, No. 3270.

The *Crataegus macracantha*, Lodd. has been superseded by *C. succulenta* Schrader, the latter name dating from 1831 while the former is usually dated from 1838, the date upon the title page of Loudon's Arb. Brit. But Lindley in the Bot. Reg. XXII under plate 1912, 1836, quotes *C. macracantha* Lodd. Cat. (without date) and Loudon, Arb. Brit. II, 819; this would indicate that that part of Loudon's work on the Crataegi had already been published; it is therefore doubtful as to which name was first published. The more familiar name is here retained. The spines of this species are very stout and heavy, often 4 inches or more long, and compound. No. 4741, October 14, 1917, Redford.

Crataegus Michiganensis, Ashe.

Collected at Redford, October 14, 1911, No. 4740; No. 3376, May 24, 1913, Belle Isle. The spines of this are very light and slender and the berries are smaller than those of the preceding; *C. macracantha* var. *minor*, Lodd., differing from the species only in the smaller fruit, may be this or more probably *C. rhombifolia* C. S. S.

DRUPACEAE.

Prunus cuneata, Raf.

On sandy hills north of Utica. Not common. No. 4496, June 10, 1911, Billington, Chandler, Farwell & Gladewitz; No. 1733, August 1, 1901, Keweenaw County.

Prunus Avium, Linn.

An escape near Walled Lake, June 5, 1911, No. 4185; Detroit, 3309, May 3, 1913; Keweenaw County, 647, August 8, 1888; Ypsilanti, 647a, May 4, 1891.

LEGUMINACEAE.

Baptisia alba (Linn.) R. Br.

In dry, rather sandy fields. Rare. No. 4492½, June 5, 1911, Walled Lake.

Cracca Virginiana, Linn.

On dry sandy hills and fields. No. 936, August 28, 1895, Orion; 936a, July 16, 1905, Island Lake; 4521, July 13, 1911, Rochester.

Lespedeza repens, Linn.

Dry sandy soils at Detroit. Rare. No. 4539, August 18, 1911.

OXALIDACEAE.

Oxalis Acetosella, Linn.

In cool, rich woods, Keweenaw County. Rare. No. 367, June 1, 1886.

Oxalis corniculata, Linn.

O. repens, Thumb.

An escape from cultivation in various places. Rochester, No. 1523, July 4, 1896. Detroit, No. 4644, September 25, 1917. Mr. Gladewitz has also found it in another section of Detroit.

Oxalis corniculata, var. *Dillenii* (Jacq.) Trelease.

O. stricta Small, Bull. Torr. Club XXIII, 267, 1896.

A common plant in southeastern Michigan. No. 4660, September 29, 1917, Dearborn; No. 4557, September 8, 1917, Royal Oak; 2712, June 16, 1912, Algonac; 2671, June 1, 1912, Parkedale; 1524a, June 14, 1901, Belle Isle; and No. 1524, July 4, 1896, Rochester. Generally slightly decumbent or erect in the spring, when it appears specifically distinct from the specific type; often creeping during the summer, and prostrate and rooting at the joints in the autumn, which proves it to be not distinct from *O. corniculata*.

Oxalis stricta, Linn.

O. cymosa Small, l. c.

Frequent in open woods and copses. Seems to be disappearing from southeastern Michigan. No. 1159, June 25, 1891, Ypsilanti; 1159a, July 21, 1892, Belle Isle; 1525, July 4, 1896, Rochester; 1525a, September 30, 1897, Belle Isle; 2813, July 14, 1912, Parkedale.

Oxalis stricta, var. *RUFA* (Small), n. comb.

O. rufa Small, Britt, Man. 577, 1901.

The plants are purplish. At Dearborn it was rather frequent and in association with the species, into which it passed. Intermediates passing through all stages of coloration from green to purple were as common as either extreme. No. 4513, July 8, 1917.

Oxalis stricta, var. *Bushii* (Small), n. comb.

O. Bushii Small, Bull. Torr. Club, XXV, 611, 1898.

Associated with the species and passing into it.

No. 2121, August 24, 1909, Geddes; No. 4543, July 29, 1917, Farmington.

PISTACIACEAE.

Rhus Canadensis, Marsh. var. *Illinoensis* (Greene) Fern.

Leaves and twigs tomentulose. Rare. Dry, sandy banks.
No. 4520, July 13, 1917, Rochester.

AQUIFOLIACEAE.

Ilex verticillata (Linn.) A. Gr. var. *tenuifolia* (Torr.) Wats.
Common in low grounds in southern Michigan. Differs from the species in being less pubescent on the lower veins of the leaves and in being pellucid punctate under a lens. No. 4716, Oxford, October 11, 1917; 3687, June 21, 1914, and 3207, October 13, 1912, Algonac; 1865, August 30, 1904, Belle Isle.

MALVACEAE.

Malva moschata, Linn.

Flowers pink, lower leaves incised. Occasional. Near Marl Lake, No. 4545, August 5, 1917.

Malva moschata, var. *undulata*, Sims.

All the leaves deeply and numerously divided into linear lobes, flowers white. Occasional, Keweenaw County, No. 364, June 1, 1886.

Hibiscus palustris, Linn.

It is more than probable that this is distinct from *H. Moscheutos*, Linn. *H. Opulifolius* Greene is probably the same as this. Grosse Isle, 4668, September 30, 1917, and 1805a, August 14, 1909; shores of Lake Erie, No. 4571½, September 9, 1917; Monroe Piers, 4570½, September 9, 1917, 1805b, August 20, 1910; Detroit, No. 1805, July 18, 1903.

UMBELLIFACEAE.

Coriandrum sativum, Linn.

On waste grounds at Detroit, No. 4391, July 18, 1893, and 2496, September 17, 1911.

Levisticum Levisticum (Linn.) Karst.

Lake Linden, where it is an escape from cultivation.

Ligusticum Canadense (Linn.) Britt.

Monroe Piers, No. 2187, August 20, 1910.

Pimpinella Anisum, Linn.

On waste grounds at Detroit, No. 1471, July 10, 1894, and 2495, September 7, 1911.

Sanicula trifoliata, Bickn.

Near Rochester, No. 4652, September 27, 1917.

GENTIANACEAE.

Gentiana linearis, Froel, var. *latifolia*, A. Gr.

A common Gentian in the Copper District of upper Michigan.

No. 292, August 1, 1885; No. 4647, September 25, 1917, Parkedale, where probably a stray introduction from further north by way of the railroad lines that pass near by.

G. flavida, A. Gr.

Occasional at Bloomfield; No. 4607, September 16, 1917.

ASCLEPIADACEAE.

Asclepias Syriaca, Linn.

In fields and waste places, common. There are two forms of this species: one, the type form, with broadly oblong or oval obtuse leaves (5-8 inches long by $3\frac{1}{2}$ inches wide) and with generally tuberculated fruits; and another form with narrowly oblong to oblong-lanceolate acute or acutish leaves (4 to 10 inches long by $1\frac{1}{2}$ to $2\frac{1}{2}$ inches wide) and with generally smooth (non-tuberculate) fruits. The first is the true *A. Syriaca*, Linn. Rochester, No. 4586, September 12, 1917; Parkedale, 2847, July 14, 1912, and 2989, August 4, 1912; 1151, Ypsilanti, June 13, 1891; 1151a, Belle Isle, July 21, 1893. The second is the var. *Illinoensis*, Pers. No. 4587, September 12, 1917, Rochester; also at Detroit.

POLEMONIACEAE.

Phlox paniculata, Linn., var. *acuminata* (Ph.) A. Gr.

Royal Oak, No. 4552, September 8, 1917; 1183, July 26, 1891,
Ypsilanti; 1151a, October 4, 1904, Keweenaw County.

Phlox subulata, Linn.

Oxford, May 20, 1917, No. 4452; Rochester, 863a, May 12,
1909; Belle Isle, 863, May 1, 1896.

BORAGINACEAE.

Symphytum officinale, Linn., var. *purpureum*, Pers.

Waste grounds at Detroit; No. 4523, July 14, 1917.

Echium vulgare, Linn.

Waste grounds at Detroit; No. 4534, July 21, 1917.

VERBENACEAE.

Lippia lanceolata, Mx.

Occasional. In the plants that I have seen the slender
peduncles, even in fruit, are shorter than the leaves, which
are often 3 or 4 inches long, lanceolate to ovate-lanceolate,
acute, coarsely dentate serrate and cuneate. No. 4798,
October 26, 1917, Rockwood; 2136, September 18, 1909,
Grosse Isle; 2136a, August 20, 1910, Monroe Piers.

LABIATACEAE.

Teucrium Canadense, Linn.

Occasional; Rockwood, October 26, 1917; No. 1298, August
12, 1892, Belle Isle; 4351, August 12, 1916, Farmington.

Teucrium occidentale, A. Gr.

Frequent; Detroit, July 21, 1917, No. 4539₂.

Teucrium menthaefolium, Bicknell.

Frequent; Monroe Piers, September 9, 1917, No. 4569; 4390,
August 20, 1916, Grosse Isle; 1877, September 13, 1904,
Detroit; 1877a, July 16, 1905, Island Lake.

Trichostema dichotomum, Linn.

Waste grounds at Detroit, rare. No. 4547, September 5, 1917.

Found also by Mr. Chandler, in, if I remember rightly, Wash-
tenaw County.

Scutellaria cordifolia, Muhl.

Banks of streams at Rochester, rare. No. 4522, July 13, 1917.

Prunella vulgaris, Linn.

Roadsides at Tecoma, No. 4635, September 23, 1917.

Prunella vulgaris, var. *Pennsylvanica* (Bigel.) Nutt.

Frequent, No. 4602, September 16, 1917, Bloomfield. Vari-
able, some of the plants bearing median stem leaves charac-
teristic of the species, the width often being 75% of the
length, the base rounded, the others being characteristic of
this variety. No. 317, August 12, 1885, Keweenaw County;
317a, July 21, 1892, Belle Isle; No. 2862, July 14, 1912,
and 3236, October 27, 1912, Parkedale.

Prunella vulgaris, var. *IODOCALYX* (Fernald) n. comb.

Prunella vulgaris var. *lanceolata* f. *iodocalyx* Fernald,
Rhodora XV, 184, 1913.

Bracts green or more or less purple and calyx purple. The
commonest form in southeastern Michigan. Nos. 4589 and
4594, September 15, 1917, River Rouge; also October 26,
1917, Rockwood.

Prunella vulgaris, var. *CANDIDA* (Fernald) n. comb.

P. vulgaris var. *lanceolata* f. *candida* Fern. l. c.

Flowers white, otherwise like the var. *Pennsylvanica*. No.
4551, September 8, 1917, Royal Oak; No. 769, July 25,
1890, Keweenaw Co.; No. 3150, September 2, 1912, Parke-
dale.

Dracocephalum Virginianum, Linn.

Occurs in abundance near Monroe Piers. No. 4566, Septem-
ber 9, 1917; Keweenaw Co., No. 621, July 26, 1888. This
is the *Physostegia Virginiana* Benth. The *Dracocephalum*
parviflorum now becomes

Moldavica parviflora (Nutt.) Britt. Keweenaw Co. Rare.
No. 1827, Aug. 1904.

The *Lycopus communis* of the "Flora" is *L. uniflorus*, Mx.

Very common in moist soils along the borders of woods, streams and in open fields. 930¹/₂, Orion, August 29, 1895; 1296¹/₂, Belle Isle, August 12, 1892; 1832, Keweenaw Co., August 1904; 3041, August 4, 1912, and 3133, September 2, 1912, Parkedale.

Lycopus uniflorus, var. *MEMBRANACEA* (Bickn.) n. comb.

L. membranacea Bickn. Britt., Man. 804, 1901.

A large-leaved shade form of *L. uniflorus*, but with thinner leaves quite different from the next variety, which has very weak, flexible leaves while those of this are firm. No. 4794, October 26, 1917, Rockwood. Mr. Gladewitz was the first to find this variety here.

Lycopus uniflorus, var. *MACROPHYLLUS* (Benth.) n. comb.

L. Virginicus var. *macrophyllus* (Bth.) A. Gr. Proc. Amer. Acad. VIII, 285, 1870.

This is the most distinct form and may be it should more properly be regarded as a distinct species. The leaves are large and weak, very thin, like tissue paper. No. 348, September 4, 1885, Keweenaw Co.

Lycopus Virginicus, Linn.

In the "Mack Woods" at Detroit. Probably a stray waif.
No. 2038, August 18, 1907.

SOLANACEAE.

Lycium Halimifolium, Mill.

This is the *L. vulgare*, a later name. Occasional as an escape. 874, October 12, 1895, Detroit; 1202, June 15, 1916, Bloomfield; 4718, October 11, 1917, Oxford.

Lycium Chinense, Mill.

Another variety of the Matrimony Vine with broader ovate leaves. An escape; Redford, No. 4446, October 11, 1917, and at Oxford No. 4717, October 11, 1917; No. 4136, October 24, 1915, Detroit.

RINGENTACEAE.

The *Ilysanthes dubia* of the "Flora" should be *I. anagalidea* (Mx.) Robinson. No. 1284, August, 1892, Belle Isle.

The *I. attenuata* is the genuine *I. dubia* (Linn.) Barnhart.

The plant as found at Detroit agrees with this in all except the fruit, which is that of the preceding; *i.e.*, the capsule is longer than the calyx. No. 4645, September 25, 1917, 1622, September 20, 1898; No. 4348, August 10, 1916, Zoo Park, Royal Oak.

Veronicastrum Virginicum, var. PURPUREUM (Raf.) n. comb.

Leptranda Virginica var. *purpurea* Ph. in Eaton & Wr. N. Amer. Bot. Ed. 8, 297, 1840.

Corollas and stamens purple; leaves in threes. Discovered by Mr. Chandler at Ypsilanti. No. 4670, September 30, 1917.

GERARDIA Authors not of Lin.

The Linnaean genus GERARDIA was adopted from Plumier and has been shown by Benthams, Pennell, and others to be a genus of the Acanthaceae. The genus Gerardia of American authors has been divided by Dr. Pennell into three genera, Aureolaria, Raf. (*Dasystoma* Authors), Temanthera, Raf. (*Otophylla* Benthams), and Agalinis, Raf. Dr. Pennell has also shown that the large yellow flowered plants that recently have been passing as species of *Dasystoma* are not of the genus of that name founded by Rafinesque, the original spelling of which is Dasistoma. The characters most relied upon, awned or awnless anthers, to separate these genera, apparently fall down, and they should be considered as congeneric. Aureolaria, the name having priority of position, should be the one to be adopted for these species.

The species found in Michigan are as follows:

AUREOLARIA, Raf. New Fl. II, 58, 1836.

Subgenus Euaureolaria (Aureolaria, Raf. l. c. in strict sense).

A. VIRGINICA (Lin.) n. comb.

Rhinanthus Virginicus Lin., Sp. Pl. 603, 1753.

A. villosa Raf. l. c. 59.

Dasystoma flava Wood, Class Book, 529, 1861.

Dry hillsides in open woods, Bloomfield, No. 4613, September 16, 1917; 1539, July 4, 1896, and 3725, July 19, 1914, Rochester; 3510, July 20, 1913, Parkedale.

A. FLAVA (Lin.) n. comb.

Gerardia flava Lin., l. c. as to description and herb.

A. glauca (Eddy) Raf. l. c. 60.

All leaves dentate to pinnatifid, the lowermost 2-pinnatifid, whole plant more or less glaucous. Bloomfield, No. 4611, September 6, 1917.

A. flava, var. INTEGRIFOLIA (Benth.) n. comb.

Dasystoma quercifolia var. *integrifolia* Benth., DC. Prodr. X, 520, 1946.

This has been referred to *A. laevigata*, Raf., but that is said to be not at all glaucous while this most assuredly is; the lowermost leaves are pinnatifid and the upper ones and those on the branches are entire. Bloomfield, No. 4612, September 16, 1917.

Sect. PEDICULARIOIDES (Benth.) (GERARDIA Sect. PEDICULARIOIDES Benth. Comp. Bot. Mag. I, 204-5, 1835).

A. Pedicularia (Linn.) Raf. l. c. 61.

The glandular form becomes

A. Pedicularia, var. AMBIGENS (Fernald), n. comb.

Gerardia Pedicularia var. *ambigens* Fernald, Rhodora X, 86, 1908.

Goodison, October 7, 1917, No. 4687; 4610, September 16, 1917, Bloomfield; 2110, August 15, 1909, Rochester; 2110a, July 7, 1916, Dead Lake; 2110b, August 27, 1910, Detroit.

SUBGENUS *Otophylla* (Benth.) (GERARDIA Sect. OTOPHYLLA Benth. l. c.).

A. auriculata (Mx.) n. comb.

Gerardia auriculata Mx. Fl. Bor. Am. II, 20, 1903.

SUBGENUS *Agalinis* (Raf.) (*Agalinis* Raf. l. c. 61).

A. ASPERA (Dougl.) n. comb.

Gerardia aspera Dougl. in DC. Prodr. X, 517, 1846.

A. PURPUREA (Lin.) n. comb.

Gerardia purpurea Lin. l. c.

A. INTERMEDIA (Porter) n. comb.

Gerardia intermedia Porter in A. Gr. Syn. II 293, 1878.

A. Gray, l. c. has forestalled the use of any other specific name for this plant by remarking that the name *intermedia* is to be adopted if the plant is to be considered of specific rank. It here includes plants that are common in Michigan and that have flowers from 10 to 12 lines long, just intermediate between the normal size for this species and those of the preceding.

A. TENUIFOLIA (Vahl) n. comb.

Gerardia tenuifolia Vahl, Symb. Bot. III, 79, 1794.

A. tenuifolia, var. ALBIFLORA (Britt.) n. comb.

Flowers white.

A. tenuifolia, var. MACROPHYLLA (Benth.) n. comb.

Gerardia tenuifolia, var. *macrophylla* Benth. l. c. 209.

A. SKINNERIANA (Wood) n. comb.

Gerardia Skinneriana Wood, Class Book 408, 1847.

CAPRIFOLIACEAE.

Viburnum Lentago. Linn.

In this and the next species the leaves are very variable as to shape and in width and glandulosity of the petioles; leaves narrowly oblong-lanceolate, narrowly elliptic, ovate, oval, or suborbicular; petioles from broadly winged and copiously glandular to wingless and glandless. Typical leaf of this species is ovate, acuminate; of the next, subrotund, crenate-serrate. Trees may be found with all the leaves of one shape only, and again leaves of all shapes may be found

upon the same tree. Possibly the variable foliage may be due to crossing. Leaves reddish or silvery scurfy, or green; branchlets and winter buds glabrous or reddish scurfy. Fruit oval or oblong, blue becoming black. 4748, October 14, 1917, Redford; 1585, September 7, 1897, Birmingham.

Viburnum Lentago var. *sphaerocarpum*, A. Gr.

Fruit spherical, smaller and retaining its bloom longer. Belle Isle, 1585a, September 15, 1897; Palmer Park, 1575b, September 24, 1897; Palmer Heights, 3955¹/₂, May 31, 1915; 4190, June 15, 1916, Bloomfield.

Viburnum Prunifolium, Linn.

Leaves smaller, less acuminate, not so inclined to be orbicular, petioles slender, generally glandless, winter buds shorter, not so acuminate, fruit oval or oblong. No. 4599¹/₂, September 16, 1917, Bloomfield; 1363, June 8, 1893, Detroit; 1363a, September 9, 1897, Birmingham; 1363b, September 15, 1897, Belle Isle.

Viburnum Prunifolium var. *globosum*, Nash.

Fruit smaller, globose. No. 4600¹/₂, September 16, 1917, Bloomfield. I have not seen any material from Michigan that will in the least correspond in its foliage to that of the eastern plant. Our plants are either a new species or new races of *V. Lentago*.

Symphoricarpus Symphocarpus (Linn.) MacM.

Near Redford, No. 4770, October 20, 1917; 733, July 7, 1890, Keweenaw County; 733a, September 18, 1909, Grosse Isle.

CAMPANULACEAE.

Campanula Americana, Linn.

Leaves oblong-lanceolate or tongue-shaped gradually tapering to each end. Rich woods. Rochester, No. 4579, September 12, 1917; 1402, July 24, 1893, Belle Isle; 1402a, September 9, 1897, Birmingham; also Redford, October 14, 1917.

Campanula Americana var. *ILLINOENSIS* (Fresen.) n. comb.

C. Illinoensis Fresen, D.C. Prodr. VII, 478, 1838.

Leaves shorter and broader, ovate or ovate-lanceolate, abruptly contracted into a winged petiole. Rich woods, rare. Rochester, 4580, September 12, 1917.

COMPOSITACEAE.

Eupatorium trifoliatum. Linn.

Lower leaves about 3 inches long, ovate or lance-ovate, acute, rounded at base, abruptly narrowed into the petiole; all the leaves *firm*, in verticills of three, rugose, pubescent and densely glandular below, scabrous above. Upper part of stem and branches of corymb *purplish, rough pubescent and glandular*. Corymb depressed. No. 4626, September 23, 1917, Tecoma; No. 778a, August 1, 1890, Keweenaw County; No. 4013½, August 26, 1915, Parkedale.

The only diagnostic character of Linnaeus is "foliis ternis."

This is the only form of the *E. purpureum* group in this section that I have found with leaves in threes.

Eupatorium trifoliatum, var. *MACULATUM* (Linn.) n. comb.

E. maculatum Linn. Amoen. Acad. IV. 288, 1755.

Similar but leaves in whorls of 4-6. Keweenaw County, No. 777, July 25, 1890; Rochester, No. 777a, July 4, 1796; Parkedale, No. 3140, September 2, 1912.

Eupatorium trifoliatum, var. *AMOENUM* (Ph.) n. comb.

E. purpureum, var. *amoenum* (Ph.) A. Gr. Syn. Fl. N. Amer. I, pt. 2, 96, 1884.

Similar but leaves mostly opposite. Tecoma, No. 4628, September 23, 1917.

Eupatorium trifoliatum, var. *FOLIOSUM* (Fern.) n. comb.

E. trifoliatum var. *foliosum* Fern. Rhodora, X, 86, 1910.

Similar but the uppermost leaves overtopping the corymb. Tecoma, No. 4629, September 23, 1917.

Eupatorium trifoliatum, var. *BRUNERI* (A. Gr.) n. comb.
Eupatorium purpureum var. *Bruneri* (A. Gr.) Robinson,
 Proc. Am. Acad. Arts and Sci. XLII, 44, 1906.

Similar to the var. *foliosum* but leaves less rugose, thinner, and the upper parts of the plant less rough and glandular. Intermediate between that and the next. Oxford No. 4766, October 16, 1917 (leaves relatively broad, coarsely few-toothed). No. 4765, October 16, 1917, Oxford, and No. 4627, September 23, 1917, Tecoma, have the leaves relatively narrow, the serratures smaller and very numerous.

Eupatorium purpureum, Linn.

The leaves in this species are very thin and flexible, larger, ovate-lanceolate elongated, (9-12 inches long and 2½-4 wide), branches of the inflorescence *canescently pubescent, not glandular*. Keweenaw County, No. 778, July 25, 1890; Rochester, 778b, July 4, 1896; Detroit, July 22, 1899.

Eupatorium purpureum var. *angustifolium*, Torr. & Gr.

Leaves very narrow. Keweenaw County, No. 441, August 6, 1886; Rochester, No. 441a, July 4, 1896; Detroit, 441b, August 24, 1904.

Artemisia Pontica, Linn.

Near Marl Lake as an escape from cultivation. The plant was not in flower but probably is of this species. No. 4544, August 5, 1917.

Artemisia Abrotanum, Linn.

An escape from cultivation. No. 4797, Rockwood, October 26, 1917, Gladewitz & Farwell. No. 823, August 30th, 1890, Keweenaw Co.; No. 3555, October 15, 1913, Detroit.

Artemisia Gnaphalodes, Nutt.

In dry grounds, not common. No. 4540, July 21, 1917, Detroit; No. 427, July 26, 1886, Keweenaw Co.

Artemisia frigida, Willd.

Banks near Dearborn. Probably an introduction by way of the railroads. Detected, I believe, by Mr. Gladewitz. No. 4527, July 14, 1917.

Centaurea maculosa, Lam.

Dry hillsides near Rochester. Gladewitz & Farwell. No. 4577, September 12, 1917.

Hypochaeris radicata, Linn.

Found at Bloomfield by Mr. Billington in 1916.

Sonchus arvensis, Linn., var. EGLANDULOSIS, n. var.

Similar to the species but destitute of glands on the involucre, pedicles, etc. Zoo Park, Royal Oak, No. 4559½, September 8, 1917.

Prenanthes alba, Lin.

Leaves oblong or lanceolate, hastate, angulate, lower 3-lobed, base subcordate, truncate, rounded, or the uppermost tapering into the petiole. No. 4680, Goodison, October 7, 1917.

Prenanthes alba, var. PINNATIFIDA, n. var.

Leaves pinnately divided into 3 to 7 lobes, lobes acute and acutely toothed, or entire on the uppermost leaves which gradually pass into the oblanceolate, entire floral leaves. Rochester No. 4671, October 7, 1917. Leaves small, the largest about 3½ inches and almost pinnate.

Prenanthes alba, var. TRILOBATA, n. var.

Leaves generally larger, often broader than long, and deeply 3-lobed, the lobes broadly lanceolate or oblanceolate and more or less toothed, the lateral ones ascending, the sinuses broadly obtuse, the upper leaves passing gradually into the entire, narrowly oblong or oblanceolate floral leaves; the lowermost leaves often have the divisions slightly 2- or 3-lobed. Goodison, No. 4679, October 7, 1917. No. 4732, Oxford, October 11, 1917. Orion, 4704, October 11, 1917.

Prenanthes alba, var. OVATA, n. var.

Median cauline leaves mostly deltoid-ovate and entire or nearly so. Goodison, No. 4678, October 7, 1917.

Prenanthes alba, var. QUERCIFOLIA, n. var.

Median cauline leaves oblong or lanceolate and coarsely 3-7 toothed. Goodison, No. 4680½, October 7, 1917. Orion, 4641, September 23, 1917.

Prenanthes altissima, Linn.

The typical form has hastately 3-lobed leaves, the lowermost sometimes pedately 5-parted. Rare Tecoma, No. 4640, September 23, 1917; Bloomfield, No. 4617, September 16, 1917; Orion, No. 918, August 29, 1895.

Prenanthes altissima, var. *CORDATA* (Willd.) n. comb.

Nabalus altissimus var. *cordatus* (Willd.) T. & G. Fl. Nor. Amer. II, 481, 1843.

Leaves mostly cordate. Common. Orion, No. 4705, October 11, 1917; Oxford, 4723, October 11, 1917; Bloomfield, No. 4616, September 16, 1917; Orion, 918b, August 29, 1895.

Prenanthes altissima, var. *DELTOIDEA* (Ell.) n. comb.

P. deltoidea Ell. Sk. II, 257, 1821-4.

Leaves mostly deltoid. Common. Tecoma, No. 4637, September 23, 1917; Orion, No. 4706, October 11, 1917; Oxford, 4728, October 11, 1917; Orion, 918a, August 29, 1895.

Prenanthes altissima, var. *OVATA* (T. & G.) n. comb.

Nabalus altissimus var. *ovatus* T. & G. l. c.

Leaves mostly ovate. Frequent. Oxford, 4722, October 11, 1917; Rochester, 3531, October 5, 1913; Detroit, 918c, September 30, 1897.

Hieracium venosum, Linn.

Frequent in dry open woods. Rochester, No. 4531, July 1, 1896; Tecoma, 4264, July 2, 1916.

Hieracium venosum, var. *NUDICAULE* (Mx.), n. comb.

H. Gronovii Linn., Sp. Pl. 802, 1753.

H. Gronovii var. *nudicaule* Mx. Fl. Bor. Am. II, 87, 1803.

H. venosum var. *subcaulescens* T. & G. Fl. N. Amer. II, 478, 1843.

With 1-3 leaves on the lower part of the stem.

Tecoma, No. 4264, July 2, 1916; Rochester, No. 4535, September 12, 1917.

This is the plant that was described by Linnaeus as *H. Gronovii*.

The plant that has been passing as such must take another name. In so far as I am able to ascertain the oldest available specific name is *subnudum*.

Hieracium subnudum (Monn.) Froel.

Stenotheca subnuda Monn., Ess. Hier. Hier. 72, t. 2, f. 2, 1829.

H. Gronovii var. *subnudum* (Monn.) T. & G. l. c., 477.

Pannicle narrow, of few heads, leaves few, mostly below the middle of the stem. Tecoma, 4264 $\frac{1}{3}$, July 2, 1916.

H. subnudum, var. FOLIOSUM (Mx.) n. comb.

H. Gronovii Monnier, l. c., 30, not of Linn.

H. Gronovii, var. *foliosum* (Mx.), l. c.

The commoner, more robust and leafy form of the species. Detroit, No. 1862 $\frac{1}{2}$, August 24, 1904.

Hieracium subnudum, var. HIRSUTISSIMUM (T. & G.) n. comb.

H. Gronovii var. *hirsutissimum* T. & G., l. c.

Similar to the last but very shaggy hirsute. This form of the species I have not seen in Michigan, but it may be expected to occur here as well as the other forms.

Hieracium paniculatum, Linn.

Found at Bloomfield by Mr. Gladewitz. No. 4614, September 16, 1917.

Hieracium scabrum, Mx.

Common in dry fields, etc. Tecoma, No. 4631, September 23, 1917; Royal Oak, Nos. 4558 and 4557 $\frac{1}{2}$, September 8, 1917; Algonac, No. 4100b, September 12, 1915; Oxford, 4770, October 11, 1917; Detroit, No. 449b, August 8, 1993, and 4410, August 27, 1916.

Hieracium scabrum, var. *tonsum*, Fernald.

A smaller form with shorter pubescence or nearly glabrous. Keweenaw County, Nos. 449, 449a, 450, 451, August 20, 1886.

Hieracium Lachenalii, C. C. Gmel.

This is the oldest available name for the polymorphous species that is passing current as *H. vulgatum* Fries; the latter name was applied to a form with relatively narrow leaves while the former represents a plant with relatively broad leaves. In the Michigan plant the root leaves are usually oblong, obtuse and apiculate, narrowed at the base, and pass gradually into the broadly ovate, acute, median stem leaves, which are on short winged petioles and about $2\frac{1}{2}$ inches long by 2 wide remotely and coarsely few dentate. Found at Bloomfield by Mr. Billington. No. 4615, September 16, 1917.

Hieracium Canadense, Mx.

In dry fields, common. Keweenaw County, No. 422, July 18, 1886; Rochester, 4654, September 27, 1917.

Hieracium Canadense, Mx. var. *MACROPHYLLUM* (Ph.)
n. comb.

H. macrophyllum Ph. Fl. II. II, 504, 1914.

A more slender, greener, and more glabrate form with larger, thinner, and broader leaves, more remotely denticulate, the lowermost often narrowed at base and becoming obovate and obtuse. Rochester, No. 4655, September 27, 1917.

Studies from the Research Laboratory.
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THE YELLOW FLOWERED CYPRIPEDIUMS.

BY OLIVER ATKINS FARWELL.

(Department of Botany, Parke, Davis & Co., Detroit, Michigan.)

ORCHIDACEAE.

CYPRIPEDIUM BULBOSUM, MILLER, GARD. Dict., No. 2, 1768.

Cypripedium parviflorum Salisb. Trans. Lin. Soc. I, 77, t. 2, f. 2 (male) 1791.

Cypripedium flavescens DC. in Redoute, Lib. I, plate 20, 1802.

In Michigan there are three distinct forms of the Yellow Ladies' Slipper: 1, with a small flower (sac $\frac{1}{2}$ –1 inch, petals $1\frac{1}{2}$ – $2\frac{1}{4}$ inches, acute); 2, with a similar flower but with sac $1\frac{1}{2}$ –2 inches long; 3, and a third, intermediate as to length of sac. In the first two the sacs are moccasin-shaped—that is, the compression is vertical, making the sac wider than deep; in the third form, the sac is bulbous, or nearly so, being slightly compressed laterally so that the sac is deeper than wide, and convex both above and below, which is not the case in the others. In the first, *the staminode is linear and obtuse*; in the 2, *oblong and obtuse*; in the 3, *deltoid and acute*. According to Dr. Rydberg (*Torreyia II*, 84–7, 1902) the second is well illustrated by Willd. in the *Hortus Berolinensis I*, pl. 13. As to general outline, therefore, but not as to size, this could pass also for the 1st form. The third is well represented by plate 911, vol. 23, of the *Botanical Magazine* and cannot be considered to be the same species. A careful analysis of the enumerated differences above outlined and a comparison of the same with the description and plate of Salisbury shows that *the plant he described had the deltoid staminode* characteristic of the third form, but the small, flat sac characteristic of the first. I have not seen any Yellow Ladies' Slipper answering to

such a combination and am forced to conclude that the drawing was made from a dried specimen of the third form in which the characteristic shape of the sac was not evident and so a conventional drawing, resembling but smaller than that of *C. Calceolus*, was made on the supposition that the sac of each was of a similar shape. This interpretation is confirmed by the statement of Dr. Rydberg that all the small-flowered forms from the eastern sections that he has seen answer to what is my third form; and by Salisbury, that his plant had been collected in Virginia by H. Marshall. In any event the deltoid, acute staminode described and figured by Salisbury places beyond doubt the identity of the form he had in hand. It is what I have outlined as the third form. In so far as my observations go, there is no variation—that is, the occurrence of intermediate forms—from the types above described. In the first two, *particularly in the first*, there is a pronouncedly sweet odor, but the third form is practically odorless; in the latter, the lateral petals are longer in proportion to the length of the sac than they are in the first two, more twisted, and not of such a deep purple color. The first specific name applied to the American Yellow Ladies' Slipper is the *Cypripedium bulbosum* of Phillip Miller. There is an earlier *C. bulbosum* of Linnaeus, but as this has been removed not only from the genus but also from the subfamily, it can not in any way interfere with the use of Miller's name. Miller, in comparing his species with the European *C. Calceolus*, says the sac of the American plant is oblong and narrower—remarks that can apply only to the sac of the third form, which also is the only one that would suggest the name *bulbosum*; his description of the lateral petals also agrees more with those of this species. *C. parviflorum* Salisb. and *C. flavesceus* DC. appear to be identical with *C. bulbosum*, Miller. No. 2615, May 30, 1912, Parkedale; No. 4171, June 6, 1916, and No. 4494, June 10, 1917, at Utica; No. 416b, June 16, 1900, Detroit.

CYPRIPEDIUM PUBESCENS, Willd. Sp. Pl. II, 113, 1805, and Hort. Berol. I, Plate 13.

No. 416, July 18, 1868, Keweenaw Co.; No. 416a, May 21, 1892, Ypsilanti; No. 3668, June 11, 1914, Rochester.

CYPRIPEDIUM PUBESCENS, Willd., var. MAKASIN, n. var.

The discussion above recorded shows that the first form, in so far as I am able to ascertain, is without a name, and I propose for it the varietal name *MAKASIN*, the Algonquin name of these flowers; and for those who recognize species only, the binominal, *Cypripedium Makasin*. The characters to be found in the staminodes appear to be constant and of good diagnostic value—*linear and obtuse* in this variety, *oblong and obtuse* in the specific type, and *deltoid and acute* in *C. bulbosum*. No. 418, July 18, 1886, Keweenaw Co.; No. 418a, May 30, 1895, Orion; No. 418b, June 1, 1910, Rochester; No. 3402, May 24, 1913, Parkedale; No. 4173, June 6, 1916, and No. 4495, June 10, 1917, Utica.

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THE TRILLIUM GRANDIFLORUM GROUP.

FARMINGTON TOWNSHIP, OAKLAND COUNTY, MICHIGAN.

BY OLIVER ATKINS FARWELL.

(Department of Botany, Parke, Davis & Co., Detroit, Michigan.)

In a certain part of Farmington Township there is a wide stretch of woodland used chiefly as pasturage for cattle. It covers several acres of ground and is very broken or hilly; a small stream winds its devious way in and out among the hills; it is of course private ground and the trespasser is fully warned not to intrude under full penalty of the law. Admission to the grounds, however, has not been denied when it has been courteously asked for. The woods are composed of oak, beech, ironwood and other deciduous trees and shrubs; in one section where the trees are scattered more than in other parts of the forest, Trilliums of the *grandiflorum* group grow in abundance. The flowers vary in color from pure white through all shades of variegation to wholly green; rose or pink flowers were conspicuous by their absence; the peduncles are ascending or erect. To the late Mr. Chandler, who died on January 17, 1918, belongs the honor of discovering this most interesting field of Trilliums. All these variable forms have been referred by authors to *T. grandiflorum*, as a rule. Rafinesque, however, published the form with shortly petioled leaves as *T. lirioides* and E. F. Smith made the long petioled form with variegated flowers a variety (*variegatum*) of *T. grandiflorum*. There is another form without leaves but with foliaceous sepals which may be an offshoot from that form, the leaves of which are long petioled; I base this opinion upon the fact that one plant of the latter had but two leaves with a very small bract in the place where the third leaf should have been; also that some of the leafless plants have, very low down on the stem, a whorl of three knobs indicating where the leaves

should have been; the knobs which I have seen have been not over $\frac{1}{8}$ inch in length and often they are merely a noticeable swelling. This form I shall name *T. Chandleri*, after the discoverer. Petals vary from ovate to oblanceolate, sessile or clawed, white, variegated, or green, large or small; flowers are usually 3 merous but may be 4 merous, and vary from single to double. The ovaries of the variegated and of the green flowers are usually green cylindrical or bottle shaped, have an evident style sometimes quite long and 3 stigmas. Whether or not they develop into fruit with viable seed I am unable to say, as I have not examined the ovules nor have I been in the field to look for fruit. The rhizomes vary from erect through all intermediate angles to complete inversion. I have observed inverted *Trillium* rhizomes in other fields as well; rhizomes often bear two stems, at least one of which, but more frequently both, bears a flower; where each stem bears a flower, they may be alike in every respect or one stem may bear a flower that is very different from that borne by the other. It is customary to consider all these forms as belonging to *T. grandiflorum*; but as they fall into one or another of three general groups I will consider each group as a species, distinguishing them as follows: *T. grandiflorum* with leaves sessile or with a short and broad, constricted base. *T. lirioides* with leaves on slender distinct petioles oftentimes several inches in length. *T. Chandleri*, *N. Sp.*, stem without leaves, sepal foliaceous. Collections were made May 19, 1917.

KEY TO THE SPECIES AND VARIETIES OF THE *Trillium grandiflorum* GROUP.

Leaves sessile, or with a short and broad, constricted base

Flowers white.

Petals obovate or oblanceolate 1½ to 2½ inches

T. grandiflorum.

Petals smaller, plant lower.

T. grandiflorum, var.
obscutum

Flowers green, petals white bordered
above the middle.

Petals orbicular, large.

T. grandiflorum, f. *orbiculare*.

Petals spatulate, large.

T. grandiflorum, f. *spatulatum*.

Flowers green throughout.

Petals obovate, large.

T. grandiflorum, f. *viride*

Petals small, ovate to oblanceolate.

T. grandiflorum, var.

obscutum, f. *viridescens*

Leaves slenderly petioled.

Petioles short, under $1\frac{1}{2}$ inches, stems long.

Petals white, small. *T. lirioides*.

Petals variegated, small to medium. *T. lirioides*, f. *albomarginatum*.

Petals green white bordered above,
large. *T. lirioides*, f. *giganteum*.

Petals green, clawed. *T. lirioides*, f. *ungulatum*.

Petioles elongated, over $1\frac{1}{2}$ inches,
stems short.

Petals white, small. *T. lirioides*, var. *longepetiolatum*

Petals variegated *T. lirioides*, var. *longepetiolatum*,
f. *variegatum*.

Petals green, white bordered at the apex. *T. lirioides*, var. *longepetiolatum*, f. *vegetum*.

Stems leafless.

Flowers white, small. *T. Chandleri*.

Flowers medium, variegated.

Flowers single. *T. Chandler*, f. *foliaceum*.

Flowers double. *T. Chandleri*, f. *plenum*.

Flowers green, white bordered at the
apex, large. *T. Chandleri*, f. *palaceum*.

Flowers green throughout.

Petals large, foliaceous. *T. Chandleri*, f. *Gladewitzii*.

Petals small, subulate. *T. Chandleri*, f. *subulatum*.

DESCRIPTIONS AND NOTES ON ABOVE MENTIONED FORMS AND VARIETIES.

Trillium grandiflorum (Mx.) Salisb. The normal form is quite common. No. 4431.

Trillium grandiflorum (Mx.) Salisb., f. ORBICULARE, N. form. Petals orbicular or nearly so, $1\frac{1}{2}$ –2 inches in diameter, mucronate, green with a white border along the edge above the middle. No. 4436. A rhizome was found with two stems each bearing a normal flower for the form.

Trillium grandiflorum (Mx.) Salisb., f. SPATULATUM, N. form. Petals spatulate-oblong to spatulate cuneate, very obtuse and mucronate, $1\frac{1}{2}$ –2 inches by $1-1\frac{3}{8}$, green with a white edge above the middle to white with a green band up and down the middle. No. 4433.

Trillium grandiflorum (Mx.) Salisb., f. VIRIDE, N. form. Petals pale green. No. 4433a.

Trillium grandiflorum (Mx.) Salisb., var. *obovatum* (Pursh) Farwell.

T. grandiflorum var. *parvum*, Gates, Ann. Mo. Bot. Gard. IV, 58, 1917.

A low form with small flowers. Frequent. No. 4443. There seem to be no distinctions between the descriptions of Pursh and Gates.

Trillium grandiflorum (Mx.) Salisb., var. *obovatum*, f. VIRESCENS, N. form. Petals, green, obovate or oblong and mucronate to ovate and ovate-lanceolate and acute, some of them clawed. No. 4443a.

Trillium lirioides, Raf. Somewhat similar to *Trillium grandiflorum* var. *obovatum* but the petals are narrower, obovate or oblong-cuneate, $1\frac{3}{4}$ inches by $1\frac{1}{2}$ inch or smaller, leaves petioled; petioles about an inch or less in length. No. 4437.

Trillium lirioides, Raf., f. ALBOMARGINATUM, N. form. Petals narrowly obovate or oblanceolate, varying from white with a green band along the midnerve to green with a white border along the edges of the upper parts. No. 4444. Of this form, 3 rhizomes (No. 4441) bearing two stems each were collected: on one of the rhizomes, one stem bore leaves only and the other was normal for the form; on another, both stems were floriferous, one stem bearing a flower of which two petals were white and the other variegated with green, and the other stem bore a flower composed of the normal parts for the form and two additional white petals, making five in all, 3 variegated and 2 white; on the third rhizome one stem was leafless but the flower had an enlarged, foliaceous calyx and 5 variegated petals, of which two were very small, while the other stem bore the usual petioled leaves, a normal calyx and nine petals, of which three are white and six variegated.

Trillium lirioides, Raf., f. GIGANTEUM, N. form. All parts much enlarged, as large as in *T. grandiflorum*; calyx foliaceous; petals spatulate-oblong very obtuse or emarginate and mucronate $1\frac{1}{2}$ – $2\frac{1}{4}$ inches long by $1\frac{3}{4}$ wide, usually sessile but occasionally with one clawed; corresponds to

forma spatulatum of *T. grandiflorum*. No. 4435. Rhizomes occasionally bearing two stems normal for the form.

Trillium lirioides, Raf., f. UNGULATUM, N. form. Petals clawed, green, ovate, obtuse and mucronate or ovate-lanceolate and acute. No. 4434.

Trillium lirioides, Raf., var. LONGEPETIOLATUM, N. var. Stem above ground short, about two inches or less, petioles usually longer than the stem, generally over two inches and a little shorter than the blades; peduncle long, erect, nearly or quite as long as the leaves, including the petioles; petals white, oblong-obovate, mucronate about 2 inches long by $\frac{3}{4}$ wide. No. 4440. Differs from the species in its short stem and long petioles, just reversing in this respect the relative proportions of these parts.

Trillium lirioides, Raf., var. *longepetiolatum*, f. VEGETUM, N. form. Petals ovate to oblong, two inches or less in length, obtuse and mucronate, green except the white margined apex, clawed. No. 4439.

Trillium lirioides, Raf., var. *longepetiolatum*, f. VARIEGATUM (E. F. Smith), n. comb.

Trillium grandiflorum, var. *variegatum*, E. F. Smith, Bot. Gaz. IV, 181, 1879.

Petals white with more or less green, chiefly along the midnerve, of variable sizes, often as large as in *T. grandiflorum*. No. 4438. One plant has a stem only an inch long and bearing two leaves each with a petiole four inches long, the third leaf being represented by a small, narrowly lanceolate bract which, including the petiole, is less than an inch long; the peduncle is seven inches long, equaling the combined length of the petiole and blade.

Trillium CHANDLERI, N. Sp. Rootstock horizontal, stem sheathed at base, for an inch or so, green, or reddish at base, six or seven inches high, leafless; sepals ovate-lanceolate or lanceolate $1\frac{3}{4}$ inches long by $\frac{5}{8}$ to $\frac{7}{8}$ wide, acute; petals white, oblanceolate or obovate-oblanceolate, shorter and narrower than the sepals. No. 4442.

Trillium Chandleri f. FOLIACEUM, N. form. Sepals enlarged and foliaceous, as in the following forms also; petals from broadly obovate to oblanceolate, oblong or ovate, generally mucronate, occasionally clawed, $1\frac{1}{2}$ to $2\frac{1}{2}$ inches long, from white with a narrow green stripe along the middle to green with only a white edge at the apex. No. 4445.

Trillium Chandleri f. PALACEUM, N. form. Petals shovel-shaped, often, including the prominent claw, 3 inches long by $1\frac{3}{4}$ wide, broadly obtuse or emarginate and mucronate, green, the upper edge bordered with white. No. 4445a.

Trillium Chandleri f. GLADEWITZII, N. form. Petals green, distinctly clawed, ovate and subrhombic, acute, foliaceous and but little smaller than the sepals, two inches long by one wide. No. 4446a.

Trillium Chandleri f. SUBULATUM, N. form. Petals green, small, claws 3-5 lines long, oblong or spade-shaped blade 10 or 11 lines long by 5 or 6 wide, abruptly contracted in to a subulate point about 3 lines long. Ovary small, white, six angled, 2 lines high, style $1\frac{1}{2}$ line long with 3 spreading stigmas of the same length. Stamens 2 with a small peduncled ovary bearing one style and stigma in the place of a third stamen. No. 4446.

Trillium Chandleri f. PLENUM, N. form. Flowers varying from partially to perfectly double. One rhizome, placed here, bore on its stalk a 4-merous flower. The petals of this form are variegated, green and white. No. 4447.



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